SEROPREVALENCE OF HIV IN PATIENTS ATTENDING VCTC IN A TERTIARY CARE HOSPITAL AND SPECTRUM OF OPPORTUNISTIC INFECTIONS AND PROFILE OF CD4 COUNTS AMONG AIDS PATIENTS AND MOLECULAR CHARACTERIZATION OF HIV.

Dissertation Submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the regulations

For the award of the degree of

M.D. (MICROBIOLOGY)

BRANCH – IV

APRIL 2014

THANJAVUR MEDICAL COLLEGE, THANJAVUR

THE TAMIL NADU Dr. MGR MEDICAL UNIVERSITY, CHENNAI, TAMIL NADU

CERTIFICATE

This is to certify that the dissertation entitled "**SEROPREVALENCE OF HIV IN PATIENTS ATTENDING VCTC IN A TERTIARY CARE HOSPITAL AND SPECTRUM OF OPPORTUNISTIC INFECTIONS AND PROFILE OF CD4 COUNTS AMONG AIDS PATIENTS AND MOLECULAR CHARACTERIZATION OF HIV.**" submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of regulations required for the award of M.D. Degree in Microbiology is a record of original research work done by Dr. K.Fatima Bathool Rani at the Department of Microbiology, Thanjavur Medical College, Thanjavur during the period from September 2012 to September 2013 under my guidance and supervision and the conclusions reached in this study are her own.

DR.K.MAHADEVAN, M.S.,
Dean
Thanjavur Medical College
Thanjavur.

DR. P.SANKAR, M.D., (Microbiology)
Signature of the Guide
Professor & Head, Department of Microbiology
Thanjavur Medical College, Thanjavur.

Thanjavur Medical College

THANJAVUR, TAMILNADU, INDIA-613004

(Affiliated to the T.N Dr.MGR Medical University, Chennai)

ETHICAL COMMITTEE

CERTIFICATE

Name of the Candidate	:	Dr. K. FATIMA BATHOOL RANI
Course	:	M.D (MICROBIOLOGY)
Period of Study	:	September 2012 – September 2013
College	:	THANJAVUR MEDICAL COLLEGE
Dissertation Topic	:	SEROPREVALENCE OF HIV IN PATIENTS ATTENDING VCTC IN A TERTIARY CARE HOSPITAL AND SPECTRUM OF OPPORTUNISTIC INFECTIONS AND PROFILE OF CD4 COUNTS AMONG AIDS PATIENTS AND MOLECULAR CHARACTERIZATION OF HIV.

The Ethical Committee, Thanjavur Medical College has decided to inform that your Dissertation Topic is approved.

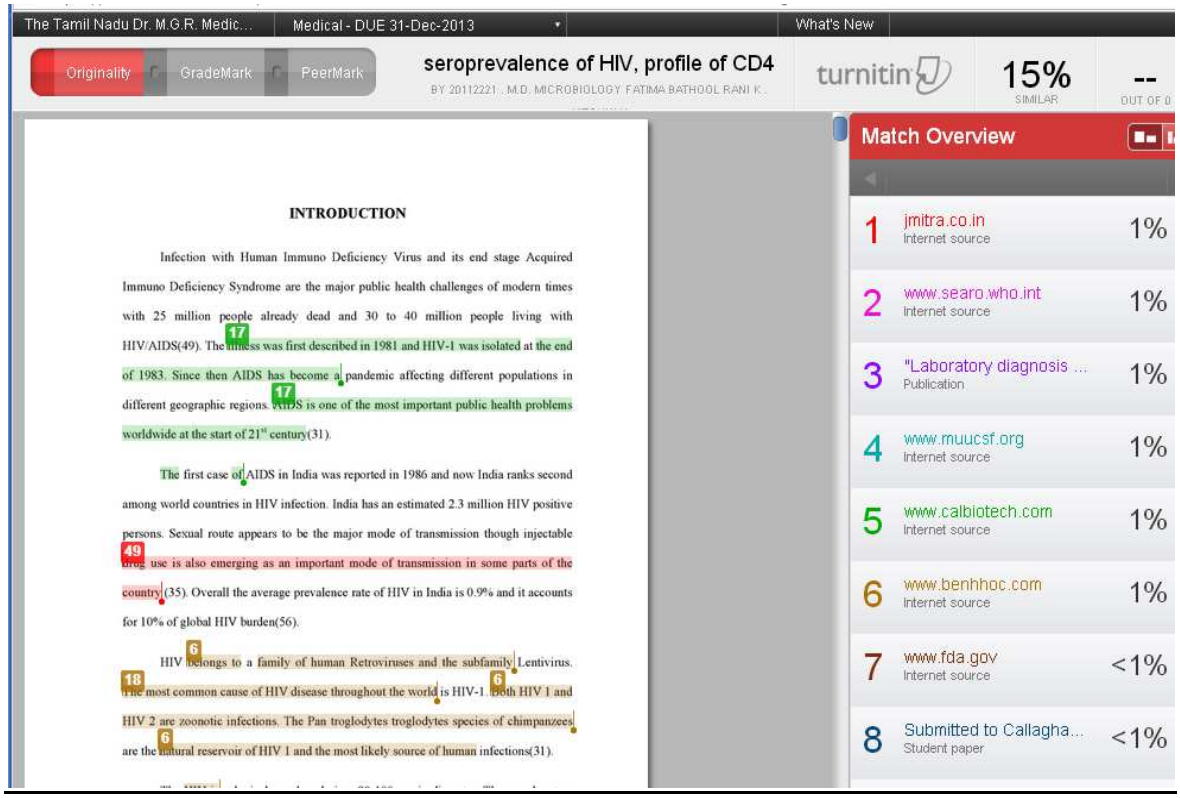
Thanjavur

Date

THANJAVUR MEDICAL COLLEGE THANJAVUR-4

Secretary

Ethical Committee


The Tamil Nadu Dr. M.G.R. Medic...
Medical - DUE 31-Dec-2013
What's New
Originality
GradeMark
PeerMark
seroprevalence of HIV, profile of CD4
BY 20112221 . M.D. MICROBIOLOGY FATIMA BATHOOL RANI K .
turnitin
15%
SIMILAR
--
OUT OF 0
INTRODUCTION
Infection with Human Immuno Deficiency Virus and its end stage Acquired Immuno Deficiency Syndrome are the major public health challenges of modern times with 25 million people already dead and 30 to 40 million people living with HIV/AIDS(49). The illness was first described in 1981 and HIV-1 was isolated at the end of 1983. Since then AIDS has become a pandemic affecting different populations in different geographic regions. AIDS is one of the most important public health problems worldwide at the start of 21st century(31).
The first case of AIDS in India was reported in 1986 and now India ranks second among world countries in HIV infection. India has an estimated 2.3 million HIV positive persons. Sexual route appears to be the major mode of transmission though injectable drug use is also emerging as an important mode of transmission in some parts of the country(35). Overall the average prevalence rate of HIV in India is 0.9% and it accounts for 10% of global HIV burden(56).
HIV belongs to a family of human Retroviruses and the subfamily Lentivirus. The most common cause of HIV disease throughout the world is HIV-1. Both HIV 1 and HIV 2 are zoonotic infections. The Pan troglodytes troglodytes species of chimpanzees are the natural reservoir of HIV 1 and the most likely source of human infections(31).
Match Overview
1 jmitra.co.in Internet source 1%
2 www.searo.who.int Internet source 1%
3 "Laboratory diagnosis ... Publication 1%
4 www.muucsf.org Internet source 1%
5 www.calbiotech.com Internet source 1%
6 www.benhhoc.com Internet source 1%
7 www.fda.gov Internet source <1%
8 Submitted to Callagha... Student paper <1%

DECLARATION

I, Dr. K .Fatima Bathool Rani solemnly declare that the dissertation entitled **"SEROPREVALENCE OF HIV IN PATIENTS ATTENDING VCTC IN A TERTIARY CARE HOSPITAL AND SPECTRUM OF OPPORTUNISTIC INFECTIONS AND PROFILE OF CD4 COUNTS AMONG AIDS PATIENTS AND MOLECULAR CHARACTERIZATION OF HIV."** submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of regulations required for the award of M.D. Degree in Microbiology, was done by me at the Department of Microbiology, Thanjavur Medical College, Thanjavur during September 2012 to September 2013. I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

Place: Thanjavur

Date: (K.FATIMA BATHOOL RANI)

ACKNOWLEDGEMENT

I am very thankful to our honorable **Dr. K.Mahadevan M.S.**, Dean, Thanjavur Medical College, Thanjavur for permitting me to carry out this study.

I express my sincere thanks to **Dr. T. Sivakami, M.S.,** Vice Principal for her kind help and encouragement during the study period.

I owe a special debt of gratitude to the honored teacher **Dr. P.Sankar, M.D.,** Associate Professor and Head, Department of Microbiology, Thanjavur Medical College for his inspiration and advice to complete this work.

I am very thankful to **Dr.S.Mini Jacob, M.D.,** Professor & HOD, Department of Experimental Medicine, The T.N. Dr. M.G.R. Medical University, Guindy, Chennai for her help in completing this study.

I am very thankful to **Dr. S. Lallitha, M.D.,** Associate Professor, Department of Microbiology, Thanjavur Medical College.

I express my sincere thanks to **Dr. D.Ayisha, M.D.**, **Dr. K.Bhuvaneswari, M.D** and **Dr. P.Shanmuga Priya, M.D.**, Assistant Professors and **Mr. D. Sivakumar, M. Sc,** Tutor for their valuable suggestions and guidance extended to me in completion of this work.

I am very thankful to **Dr. D. Neelaveni, M.D.,** Assistant Professor, Salem Medical College, Salem.

I would like to thank all my Co- PGs for their kind support and Co-operation. I am very thankful to all Laboratory Technicians for their help extended by them.

I wish to extend my thanks to all my family members especially my mother for their sacrifices and constant support during the entire period of this study.

I also extend my thanks to all the patients who participated in my study.

CONTENTS

LIST OF ABBREVIATIONS

AIDS	-	Acquired Immuno Deficiency Syndrome
CRF	-	Circulating Recombinant Forms
CMV	-	Cytomegalo Virus
EBV	-	Epstein Barr Virus
ELISA	-	Enzyme Linked Immuno Sorbent Assay
HIV	-	Human Immunodeficiency Virus
HRP	-	Horse Radish Peroxidase
HSV	-	Herpes Simplex Virus
HTLV	-	Human T cell Lymphotropic Virus
IDU	-	Injectable Drug Use
LAV	-	Lymphadenopathy Associated Virus
NACO	-	National AIDS Control Organisation
NACP	-	National AIDS Control Programme
RT-PCR	-	Real Time Polymerase Chain Reaction
TMB	-	Tetra Methyl Benzidine
TNF	-	Tumour Necrosis Factor

LIST OF TABLES

LIST OF COLOR PLATES

SEROPREVALENCE OF HIV IN PATIENTS ATTENDING VCTC IN A TERTIARY CARE HOSPITAL AND SPECTRUM OF OPPORTUNISTIC INFECTIONS AND PROFILE OF CD4 COUNTS AMONG AIDS PATIENTS AND MOLECULAR CHARACTERIZATION OF HIV.

ABSTRACT

Introduction :

Infection with Human Immuno Deficiency Virus and its end stage Acquired Immuno Deficiency Syndrome are the major public health challenges of modern times. The HIV positive patients are extremely susceptible to a variety of opportunistic infections which cause morbidity and hospitalization. Though curative treatment for HIV is not available at present, we can minimize the HIV infection by early screening and health education.

Aim & Objectives :

To study the seroprevalence of HIV infection in Thanjavur by subjecting the serum samples to Rapid Card tests and confirm by ELISA. To determine the CD4 counts of the reactive patients. To categorise the cases according to the presenting complaints and screen for Opportunistic Infections and do Molecular Characterisation for HIV-1.

Materials & Methods :

All cases were screened by COMB-AIDS kit at VCTC, TMCH, Thanjavur. Those samples which test reactive to COMB-AIDS kit are subjected to HIV Triline, HIV Trispot & ELISA.CD4 counts of the reactive patients were detected. Zeihl – Neelsen staining of sputum, culture of oral swabs for Candida, Toxoplasma, HSV-2

screening by ELISA, Cryptococcal latex agglutination test were done for reactive cases.Molecular Characterisation of HIV-1 was done for 10 samples.

Results :

Seroprevalence of HIV was 2.8%.Oral Candidiasis (39.02%) emerged as the most common Opportunistic Infection followed by Pulmonary Tuberculosis (28.03%), Herpes Simplex Virus -2 (14.45%) Toxoplasmosis (5.78%) and Cryptococcosis (3.41%). All 10 samples answered positive in PCR.

Keywords:

HIV, Seroprevalence, CD4 Count, Opportunistic Infections.

INTRODUCTION

Infection with Human Immuno Deficiency Virus and its end stage Acquired Immuno Deficiency Syndrome are the major public health challenges of modern times with 25 million people already dead and 30 to 40 million people living with HIV/AIDS(49). The illness was first described in 1981 and HIV-1 was isolated at the end of 1983. Since then AIDS has become a pandemic affecting different populations in different geographic regions. AIDS is one of the most important public health problems worldwide at the start of 21st century(31).

The first case of AIDS in India was reported in 1986 and now India ranks second among world countries in HIV infection. India has an estimated 2.3 million HIV positive persons. Sexual route appears to be the major mode of transmission though injectable drug use is also emerging as an important mode of transmission in some parts of the country (35). Overall the average prevalence rate of HIV in India is 0.9% and it accounts for 10% of global HIV burden(56).

HIV belongs to a family of human Retroviruses and the subfamily Lentivirus. The most common cause of HIV disease throughout the world is HIV-1. Both HIV 1 and HIV 2 are zoonotic infections. The Pan troglodytes troglodytes species of chimpanzees are the natural reservoir of HIV 1 and the most likely source of human infections(31).

The HIV is spherical enveloped virus 80-100 nm in diameter. The core has two single stranded positive sense RNA, Reverse transcriptase, Protease and Integrase enzymes. The genome has three structural genes gag, pol and env and six non- structural proteins Vif, Vpu, Vpr tat, rev and nef(37).

After entering a person's body, HIV infects the cells and starts to replicate in the CD4 T cells and macrophages. It induces the body's immune system to produce antibodies specific to HIV. The period between acquisition of infection and production of detectable HIV antibodies is called window period lasting for 2 to 12 weeks. During this period the person is highly infectious but may not test positive for common HIV antibody tests (56).

At the time of infections 30 to 50% of persons have a recognizable acute illness. Symptoms of acute HIV infections include fatigue, rash, headache, nausea and night sweats. Because of the progressive destruction of CD4 lymphocytes and other immune cells, there is decline of immune response, patients with HIV are prone to develop a panorama of diseases during their life time (37).

The HIV positive patients are extremely susceptible to a variety of opportunistic infections (35). These are called opportunistic infections because the advantage of the opportunity offered by a weakened immune system. Since the beginning of HIV epidemic, opportunistic infections have been recognized as common complication of HIV infection. Opportunistic infections cause substantial morbidity and hospitalization, necessitate toxic and expensive therapies and shorten the survival of people with HIV infection(14,34, 82).

In India TB is the most common opportunistic infection among the HIV infected individuals. Other commonly reported opportunistic infections include Oral Candidiasis, Herpes zoster, Cryptococcal meningitis, Cerebral toxoplasmosis and Cytomegalovirus retinitis(50).

The introduction of ART has dramatically reduced the incidence of opportunistic infections among the HIV infected individuals(61). However most of the people are living in areas with limited access to ART. The initiation of primary prophylaxis for opportunistic

infections is based chiefly on CD4 count which has shown to be an excellent predictor of short term overall risk of developing AIDS among HIV infected patients(56).

HIV infected people may remain asymptomatic for as long as ten or more years. People in this phase potentially play an important role in the transmission of HIV. They are infectious and cannot be identified by screening their serum for HIV antibodies (37).

The lab diagnosis of HIV infection is based on the detection of HIV antibodies. A variety of HIV antibody assays are available like ELISA, Western blot and Rapid tests. Rapid tests are most appropriate for smaller health institutions. They are quicker and they do not require special instrumentation or training (56).

HIV infection cannot be successfully diagnosed during the window period using antibody based assays. Assays which detect the virion are used in this phase. The tests employed are p24 antigen and HIV proviral DNA assays. The proviral DNA assays is based on PCR which is highly sensitive and specific (56).

Health education and case detection are presently the only ways to combat the catastrophe. Information, Education, Communication create awareness among general population(35).Hence I have undertaken this project to screen the patients for HIV infection and assess the clinical profile with respect to the presenting symptoms, CD4 count so that Opportunistic infections can be diagnosed early and treated thereby quality and expectancy of life can be improved.

AIM & OBJECTIVES :

1. To study the demographic profile of HIV in Thanjavur district, Tamil Nadu by screening the patients attending ICTC, TMCH, Thanjavur.
2. To study the seroprevalence of HIV infection in Thanjavur by subjecting the serum samples to Rapid Card tests.
3. To confirm the positive cases by ELISA.
4. To determine the CD4 counts of the reactive cases.
5. To categorise the cases according to the presenting complaints and screen for Opportunistic Infections.
6. To study the molecular characterisation of HIV by RT-PCR.

REVIEW OF LITERATURE

HISTORICAL REVIEW :

HIV/AIDS is quite recently detected disease. This disease found its gateway in the mid 20th century. HIV is supposed to have spread from chimpanzees to humans in Africa. The virus is transmitted to humans following contact with blood of infected chimpanzees during hunting (49).

For a long time HIV was present at low levels in equatorial Africa in 1950s, 1960s, and early 1970s. A young Norwegian sailor travelled to African ports and he became ill. He transmitted the illness to his wife and his new born daughter. A Portugese man who visited Africa developed similar illness in 1974 (67).

In 1981, five homosexual men with Pneumocystis jiroveci pneumonia were reported in Los Angeles(52). Kaposi's sarcoma was reported in 26 homosexuals in New York & Los Angeles. The disease was named GRID (Gay Related Immuno Deficiency). Later the same illness was found among Injectable Drug Users and Blood transfusion recipients (31).

The etiological agent was named LAV(Lymphadenopathy Associated Virus) in France and HTLV-III in USA. International Committee for the Taxonomy of viruses recommended the current designation HIV(Human Immuno-deficiency Virus). In 1986, Zidovudine was developed. December 1, 1988 marked the first world AIDS day (6, 67). In 1991, dideoxycytidine was developed. In 1993, HIV first developed resistance to Zidovudine. In 1996, Nevirapine was approved. In 2000, trials for HIV vaccine started in Oxford. In 2003, Enfuvirtide was approved for HIV.

GLOBAL SCENARIO :

Globally, 33.2 million people are currently living with HIV of which 30.8 million are adults and 2.5 million are children below 15 years. Among the 30.8 million, 15.4 million are women amounting to 50% of infections. 2.5 million people are newly infected every year with HIV of which 2.1 million are adults and 0.4 million are children(31).

The number of deaths due to AIDS continues to be alarmingly high and 2.1 million lost their lives due to AIDS in 2008 of which 1.7 Million are adults and 0.4 million are children. (67)

INDIAN SCENARIO :

The first case of HIV in India was diagnosed in Govt. General Hospital, Chennai by Dr. Suniti Solomon in 1986(70). The patient was a Commercial Sex Worker from Mumbai. The seroprevalence of HIV is high in south and north-east. There is a great geographic variation of HIV infection in India.

About 2.3 million people are living with HIV of which 39.3% are women and 60.7% are men, 86.5% are in the age group of 15-49 and 13.5% are less than 15 years(32, 78).

The epidemic is moving to general population from high risk group and to rural areas from urban. A wide variation is seen between states and even districts (32, 78). In South, the major mode of transmission is heterosexual whereas in North, it is IV drug abuse(32).

TAMIL NADU SCENARIO (56):

About 2.1 lakhs people are living with HIV. The HIV prevalence among

1. Ante Natal Cases- 0.25%
2. Injectable Drug Users- 16.8%
3. Men having Sex with Men- 6.6%
4. Female Sex Workers – 3.6%

Namakkal is the worst affected district with the seroprevalence of 4.4% in 2003 and 2.8% in 2004 because of its large trucking industry followed by Perambalur.

NACP-III (National AIDS Control Programme - III) classified the districts into 4 categories A, B, C, D based on HIV prevalence (46).

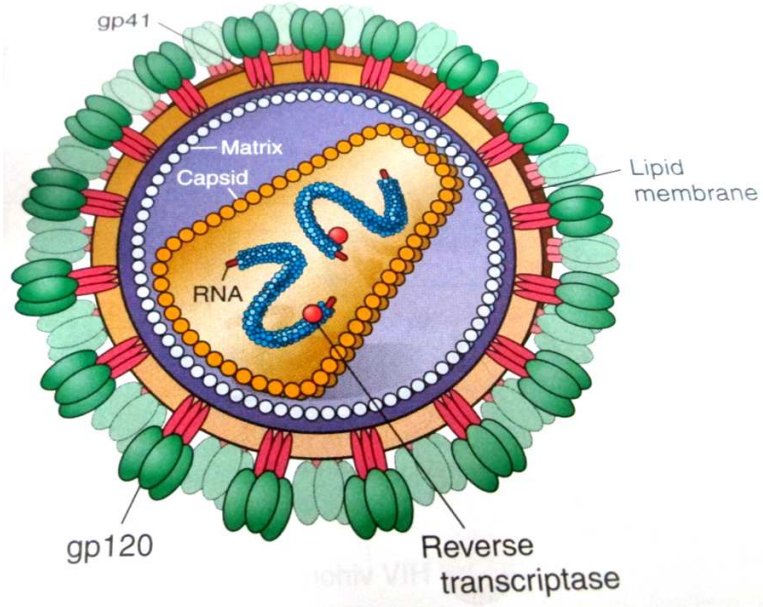
1. Category A – districts with > 1% ANC prevalence during the last 3 years.
2. Category B – districts with < 1% ANC prevalence during the last 3 years, with > 5% population in High Risk Group
3. Category C – districts with < 1% ANC prevalence during the last 3 years, with < 5% population in High Risk Group with known hot spots (Migrants, Truckers, Factory Workers & Tourists).
4. Category D – districts with < 1% ANC prevalence during the last 3 years, with < 5% population in High Risk Group with no known hot spots.

TN has 30 districts of which 22 districts come under Category A, 5 under Category B and 3 under Category C.

MORPHOLOGY OF HIV (24, 31, 49):

The etiological agent of AIDS is Human Immunodeficiency Virus which belongs to the family Retroviridae and subfamily Lentivirus.

Electron Microscopy shows that HIV virion has icosahedral symmetry. The mature infectious virus particle buds from the cell membrane. It is spherical in shape. It has an outer lipid bilayer and a nucleocapsid and the core is cone shaped. One end of the core is broad and the other end narrow.



The outer membrane has 72 knobs. The knobs are assembled as trimers with two envelope proteins gp120 and a transmembrane protein gp41. The viral membrane is cholesterol rich and incorporates a variety of host proteins like MHC Class I and Class II.

The nucleocapsid has two molecules of single stranded RNA surrounded by three proteins which are products of gag gene. They are

1. P 17(17kDa) – matrix protein
2. P 24(24 kDa)- forming nucleo capsid shell
3. P7, p6 - nucleoproteins tightly bound to viral RNA

The core also has enzymes protease, Reverse Transcriptase, Integrase, t RNA and Vpr.

HIV GENOME (39):

The genome has various overlapping open reading frames coding for several viral proteins. It is 9.7 kb in length and has three genes gag, pol, env followed by two complete Long Terminal Repeats. The 5' end begins with

1. Gag gene – coding for four proteins p17, p24, p7, p6 followed by
2. Pol gene – coding for Reverse Transcriptase (p66/51), Integrase(p32) and Protease(p10) followed by
3. Env gene – coding for polyprotein gp160 which is then cleaved into proteins gp120 and gp41 constituting the outer membrane of the virus(82).

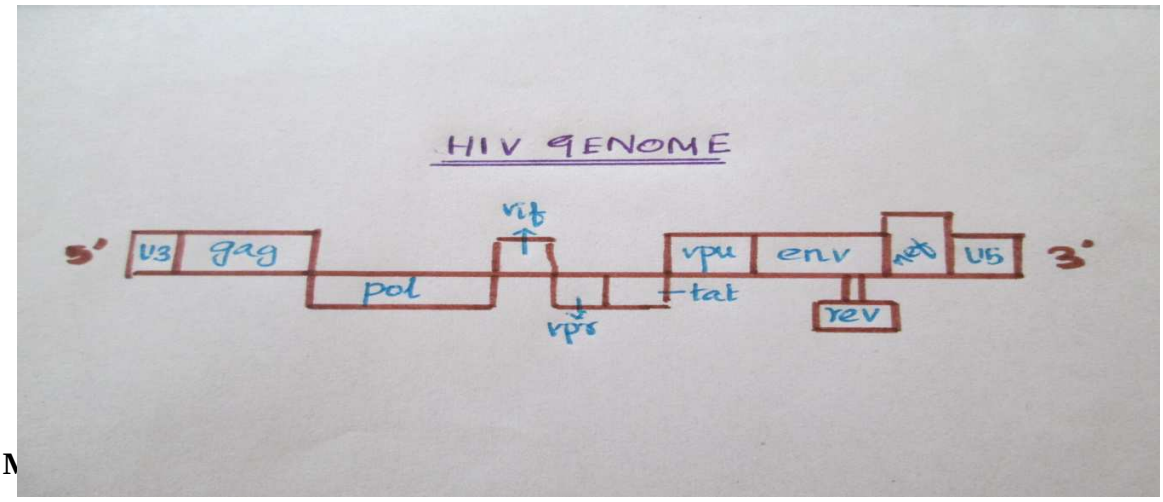
HIV also has six non- structural proteins like Tat, nef, rev, vif, vpu

And Vpr. Between pol and env genes are located

1. Vif – Viral Infectivity Protein – increases the infectivity of viral particles and degradation of viral DNA.
2. Vpr – Viral protein r – enhances viral replication
3. Tat – Transactivator of Transcription – major viral transactivator and produces immune suppression.
4. Rev – regulator of expression of viral proteins and viral RNA

5. Vpu – Viral protein u – enhances virion release and CD4 degradation. Located downstream from env gene is
6. Nef – Negative Regulatory Factor – inhibits or enhances viral replication depending on the cell infected; anti apoptotic.

The major difference between HIV-1 and HIV-2 is that HIV-2 lacks Vpu and has Vpx(42).



There are two major lentiviruses – HIV-1 and HIV-2. There are three major groups of HIV-1.

1. Group M- Major – responsible for most of the infections
2. Group O – Outlier – found in Cameroon, Gabon
3. Group N – rare(33).

HIV-1 is more closely related to viruses isolated from chimpanzees pan troglodytes troglodytes which are the natural reservoir of HIV-1. HIV-2 causing infections sporadically is more closely related to Cameroonian Gorillas (55).

The M group has nine subgroups or clades A, B, C, D, F, G, H, J, K and there are minor circulating recombinant forms (CRFs)(39). The prevalent CRF is CRF01_AE which is prevalent in South east Asia followed by CRF02_AG which is common in West and Central Africa.

Subtype/ Clade B is seen in USA, Canada, Western Europe and Australia. Subtype/ Clade C is the most common form worldwide and in India and is responsible for more than 50% of the infections. Usually an individual is affected by viruses of more than one subtype. Among HIV-2 there are five genotypes HIV2A to E. This molecular heterogeneity of HIV is due to the error prone nature of Reverse Transcriptase.

MODES OF TRANSMISSION OF HIV (31, 41, 67):

1. Unprotected sexual intercourse(heterosexual, homosexual)

2. Parenteral(use of inadequately sterilised syringes, needles)

3. Perinatal (From mother to child during pregnancy, delivery and breast feeding)

SEXUAL TRANSMISSION :

75 to 80% of HIV infection in adults are transmitted through unprotected sexual intercourse. (75) (Heterosexual – 70% which is common in developing countries, Homosexual – 5 to 10% common in North America, Europe, Australia).

There is strong association between anal intercourse and HIV as there is only a thin, fragile rectal mucous membrane separating semen from the cells which are the targets for HIV (75). Infection with microorganisms like Treponema pallidum, Haemophilus ducreyi, Neisseria gonorrhoea, Chlamydia, HSV, Trichomonas vaginalis cause genital ulcers which increase the

risk of transmission of HIV. Treating Sexually Transmitted Diseases reduce the risk of transmission of HIV.

PARENTERAL TRANSMISSION :

Parenteral transmission during IV drug use does not require i.v. puncture. Even subcutaneous/ intramuscular injections also transmit infections. The sharing of infected needles transmit about 5-10% of infections. It is the dominant mode of transmission in developed countries and in developing countries blood transfusion is the cause (1). This can be decreased by screening the blood for viral nucleic acid, p24 antigen and antibodies.

Health Care Workers develop HIV by percutaneous injuries or by contact of infected material with non intact skin or mucous membrane. The risk of transmission of HIV by percutaneous injuries is 0.3% and by mucocutaneous injuries is 0.09%.Factors associated with mucocutaneous transmission are exposure to large volume of blood, increased duration of contact with the infected material.

HIV can be transmitted by CSF, Synovial fluid, Pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid. Faeces, urine, tears, nasal creations, sweat saliva, sputum and vomitus are not infectious if they are not bloody.

PERINATAL TRANSMISSION:

Factors associated with perinatal transmission are advanced maternal stage, increased viral titres, decreased vitamin A levels, choriaamnionitis, maternal anaemia, placental malaria etc., In the absence of prophylactic ART, HIV can be transmitted from the mother to fetus in the first trimester. When the viral load is less than 1000 copies/ml, HIV is not

transmitted to fetus but when the load increases to more than 1 lakh copies/ml, the rate of transmission increases to 40%.

Zidovudine, when given to the mother from II trimester reduces the rate of transmission. Nevirapine given at the onset of labour to the mother, to the newborn within 72 hours of birth decreases the transmission rate. Caesarean delivery and giving artificial feeds also decrease the transmission.

LIFE CYCLE OF HIV (31) :

The replication cycle of HIV consists of the following steps

1. Attachment
2. Penetration
3. Uncoating
4. DNA synthesis
5. Nuclear transport
6. Integration
7. Particle Assembly and Budding.

ATTACHMENT :

The replication cycle begins with attachment of V1 region of gp120 to CD4 receptors and coreceptors CXCR4 and CCR5. This attachment produces a confirmational change of gp120 and it releases gp41 from high energy configuration. CXCR4 is the coreceptor for T cell tropic, synctitium forming viruses – X4 viruses, which are responsible for sexually transmitted infections. CCR5 is the coreceptor for macrophage tropic, non synctitium forming viruses – R5

viruses, which are the dominant types in the later stage of disease resulting in rapid progression of the disease and death of the AIDS patient.

PENETRATION :

Gp41 fuses with host cell membrane and penetrates the plasma membrane of the target cell and brings the virion and target cell together.

UNCOATING :

Uncoating occurs by phosphorylation of viral matrix protein by MAP kinase. Nef causes changes in pH and promotes uncoating. At the end of uncoating viral RNA and enzymes are released from the cell membrane.

DNA SYNTHESIS :

As the viral particles traverses the cytoplasm and reaches the nucleus the viral Reverse Transcriptase catalyses the transformation of single stranded viral RNA into double stranded cDNA. The protein coat releases the DNA into the cytoplasm. In the viral RNA the arrangement is

LTR- 5'- gag,pol,env- 3'-LTR

In the viral DNA the arrangement is as follows

U3-LTR- U5- gag,pol,env- 3'-LTR- 5'

NUCLEAR TRANSPORT :

The double stranded DNA or the Pre- Integration Complex is 28nm in radius which is twice the radius of nuclear pore. So this transport requires Nuclear Localisation Signals located in the viral proteins especially vpr which disrupts the nuclear envelope and helps in the entry of Pre- Integration Complex into the nucleus.

INTEGRATION :

Viral double stranded DNA is inserted into host cell genome and provirus is formed. This reaction is catalysed by Integrase. This removes the terminal nucleotides from the DNA and inserts in the host genome.

ASSEMBLY AND BUDDING :

Viral proteins are assembled from outermost to innermost p17, p24, Protease, Reverse Transcriptase, Integrase. The nucleoprotein helps in encapsidation. Then gp160 cleaved in Endoplasmic Reticulum and Golgi Apparatus into gp120 and gp 41.

Budding occurs in special regions of lipid layer called lipid rafts where the Nucleocapsid acquires the envelope. Protease catalyses the cleavage of gag and pol precursors and yields mature virion.

IMMUNE RESPONSE (31) :

Both Humoral and Cell mediated immunity play an important role in HIV infection.

Humoral Immunity :

1. Binding antibody

2. Neutralising antibody – type and group specific
3. ADCC antibody
4. Enhancing antibody
5. Complement

Cell mediated Immunity :

1. CD4 Helper T cells
2. CD8 Cytotoxic T cells
3. Natural Killer cells
4. ADCC

Humoral Immune Response :

Antibodies appear within 6 to 12 weeks of infection. These are the binding antibodies detected by ELISA & Western Blot assay. The first antibodies detected are those formed against gag gene proteins p17 & p24 followed by antibodies to env gene proteins gp160, gp120, gp41 and pol proteins p31, p51 and p66. Most of the neutralizing antibodies are formed against the hypervariable region of gp120 protein which is known as the V3 loop region.

Antibodies which mediate Antibody Dependent Cell mediated Cytotoxicity and antibodies directed against gp41 also help infection of cells by HIV and this phenomenon is known as **antibody enhancement**. Certain antibodies kill the uninfected also along with infected cells and this is known as **bystander killing.** The infected cells are killed by Complement also.

Cellular Immune Response :

This is mediated by CD4 Helper T cells and CD8 Cytotoxic T cells. Though CD4 cells are the targets of HIV, they undergo proliferation and secrete IL-2 & IFN-γ. CD8 cells produce perforins and cause destruction of HIV infected cells bearing class I MHC molecules.CD8 cells inhibit the replication of HIV and this is mediated by chemokines MIP- 1α and MIP-1β.

PATHOGENESIS OF HIV:

The main pathogenesis of HIV is the marked immune deficiency caused by destruction of CD4 Helper T cells (62). When the virus is transmitted by infected blood, contaminated needles and from mother to fetus, it enters the spleen from the circulation where it forms the primary focus. From there it goes to other lymphoid organs like GALT- Gut Associated Lymphoid Tissue producing **initial viremia**.

When HIV is transmitted via sexual intercourse and infected breast milk then dendritic cells play a major role in the pathogenesis. These cells express a receptor DC-SIGN on their surface which binds to gp120 of HIV and trap the HIV particle within them. They retain the HIV particle for many days and mediate transinfection of CD4 cells.

The virus starts replicating in the CD4 cells before specific immune response is mounted. This results in widespread dissemination of virus to brain and other tissues. The virus evades the immune system and starts replicating continuously for about ten years thus setting up **chronic, persistent infection**.

CD4 count starts to decline but the patients are asymptomatic and this is known as clinical latency but there is no microbiological latency as there is always some level of virus is

replicating. If the patients are not treated CD4 count starts progressively falling down and the patient becomes susceptible to opportunistic infections.

LONG TERM SURVIVORS & LONG TERM NONPROGRESSORS :

The patients living for more than 20 years after initial infection are long term survivors. They may have immuno deficiency, opportunistic infections even very low CD4 count. In spite of these they survive for more than 20 years. This may be due to ART and prophylaxis against opportunistic infections.

The patients who have HIV infection for more than 10 years with normal CD4 counts and who are stable without ART are long time non progressors. This may be due to efficient humoral and cell mediated immune responses against the viruses.

NATURAL COURSE OF HIV (41) :

The course of HIV is defined three phases which occur over a period of 10 years which include :

1. Primary HIV infection
2. Chronic asymptomatic phase
3. Overt AIDS

Primary HIV infection :

This stage is characterised by initial rise in plasma viremia to more than 1million copies/ml, decrease in CD4 cells in blood and tissues and increase in blood CD8 cells. Virus specific immune responses are mounted resulting in decrease of plasma viral load. This causes

resolution of clinical syndrome.HIV antibodies are negative and so the diagnosis is based on p24 assay and the detection of plasma viral RNA.

Chronic asymptomatic phase :

The first phase is followed by the phase of clinical latency. In this phase the viral load and CD4 counts are stable. The virus replicates at low levels in the gut and they are trapped in the Follicular Dendritic Cells in the lymphoid tissue. The ability of the immune system to maintain effective and specific immune responses is impaired. As a result of this the levels of viremia rapidly rise and CD4 count fall progressing towards the advanced stage with constitutional symptoms and opportunistic infections.

Overt AIDS :

This is the end stage of HIV infection leading to death in 2-3 years in the absence of ART. CD4 falls below 50cells/ml.

CLINICAL MANIFESTATIONS OF AIDS :

Early Stage :

This stage is usually asymptomatic or the patient may experience mild flu like symptoms. This acute clinical syndrome is characterised by fever, fatigue, arthralgia, headache, rash, lymphadenopathy, pharyngitis, myalgia, night sweats, gastrointestinal disturbances, oral/genital ulcers and the CD4 counts are above 500cells/ml. In 80-90% of patients the illness is very mild that they do not seek medical attention.

Intermediate Stage :

This stage is also asymptomatic or without serious manifestations. CD4 counts are between 200-500 cells/ml. Skin and mucosal infections are common. Herpes simplex, Herpes zoster, Oral/vaginal Candidiasis, Oral Hairy Leucoplakia, respiratory tract infections caused by Streptococcus pneumoniae, Pelvic Inflammatory Disease occurs in this stage. Patients experience fever, diarrhoea and weight loss. These manifestations are referred to as " AIDS Related Complex."

Late Stage :

This stage is defined by CD4 count below 200 cells/ml and presence of AIDS defining illness.

WHO Classification System for HIV Infection (67) :

Clinical Stage I

1. Asymptomatic Infction
2. Persistent Generalised Lymphadenopathy
3. Acute Retroviral Infection

Performance Stage I

Asymptomatic, normal activity level

Clinical Stage II

1. Unintentional Weight loss < 10% of body weight
2. Minor mucocutaneous manifestations (dermatitis, angular cheilitis, fungal nail infections)

3. Herpes zoster within last 5 years
4. Recurrent upper respiratory tract infections

Performance Stage II

Symptomatic but fully ambulatory

Clinical Stage III

1. Unintentional Weight loss > 10% of body weight
2. Chronic diarrhoea > 1 month
3. Fever > 1 month
4. Oral Candidiasis
5. Oral Hairy Leucoplakia
6. Pulmonary TB
7. Severe bacterial infectons
8. Vulvo vaginal Candidiasis

Performance Stage III

In bed more than usual

Clinical Stage IV

1. HIV wasting syndrome
2. Pneumocystis jiroveci pneumonia
3. Toxoplasmosis of brain
4. Cryptosporidiasis with diarrhoea > 1 month
5. Isosporiasis with diarrhoea > 1 month

6. Cryptococcosis, extrapulmonary
7. Cytomegalovirus infection of an organ other than liver, spleen or lymph node
8. Herpes Simplex infection, mucocutaneous
9. Progressive Multifocal Leucoencephalopathy
10. Any disseminated endemic mycosis
11. Candidiasis of esophagus, trachea, bronchus, lung
12. Atypical Mycobacterial infection
13. Non typhoid Salmonella septicaemia
14. Extrapulmonary TB
15. Lymphoma
16. Kaposi's Sarcoma
17. HIV encephalopathy

Performance Stage IV

In bed > 50% of daytime

OPPORTUNISTIC INFECTIONS (31,41) :

These infections are named so as they take the advantage or opportunity of weakened immune system. These infections are the major causes of mortality and morbidity in HIV.

TUBERCULOSIS (17, 41) :

HIV infection is an important risk factor for the development of Tuberculosis. It increases the risk of activation of latent TB and also the risk of primary disease following exogenous

infection. In India there are a large number of new TB cases and 5% of these are coinfected with HIV.HIV patients with TB are less infectious as they have only non-cavitating lesions.

CD4 cells play a major role in the immunity against TB including the formation of epithelial granulomas which restrict the growth of the organism and these cells are very much depleted in HIV infection which in turn facilitates the fluorishing growth of M.tuberculosis(59). Host immune response to M.tuberculosis promotes HIV replication. So the both microorganisms mutually help each other.

Sites of TB disease like granulomas create favourable environment for HIV replication. Host responses against M.tuberculosis like secretion of proinflammatory cytokines like TNF, activation of mononuclear cells in turn favours tremendous replication of HIV. So both HIV and TB infections go hand in hand.

Clinical features of TB in HIV patients with normal CD4 counts are similar to HIV negative individuals with TB(85). With progressive fall of CD4 counts atypical presentation of pulmonary TB, sputum smear negative TB, extrapulmonary TB, miliary TB predominate. Cervical, Inguinal, mediastinal lymphadenopathy, pleural, pericardial effusions, abscesses in liver, spleen are also common.

Candidiasis :

Oral Candidiasis is seen in most of the HIV patients, esophageal Candidiasis in advanced stage and vulvovaginal Candidiasis in one fourth of the women. Most of the infections are caused by C.albicans. The most common presentation is creamy white patches on an erythematous base. Patients with oesophageal Candidiasis experience difficulty in swallowing

whereas in vulvovaginal Candidiasis patients complain of vaginal discharge, itching and burning pain.

Oral Candidiasis in HIV patients is a sign of progressive immune Deficiency(41,71). ART should be started along with anti fungal therapy like fluconazole or itraconazole. Esophageal Candidiasis requires systemic therapy. If ART is not started, relapses are common.

Herpes Simplex infection :

Herpes reactivation occurs in advanced disease when CD4 falls below 100. It is characterised by painful ulcers at mucocutaneous junctions followed by ulcerations. Recurrent genital, perirectal ulcers are common due to reactivation of HSV-2.Most infections are treated with oral aciclovir for 5-14 days and severe mucocutaneous infections are treated with intravenous aciclovir. Herpes zoster can occur at any stage of HIV infection. The lesions are bullous or haemorrhagic and severely painful along a single dermatome.

Toxoplasmosis :

This is common in advanced disease when CD4 count falls below 100 cells/ml. This is caused by Toxoplasma gondii, an obligate intracellular parasite. It occurs as a result of activation of latent T.gondi. CNS is the common site of Toxoplasmosis. Toxoplasmic encephalitis occurs as single or multiple intracranial abscesses with focal neurologic signs and constitutional symptoms. Fever, headache, confusion, lethargy, seizures are common. Diagnosis is by MRI and ELISA for anti-toxoplasmal antibodies. Treatment is a combination of Pyrimethamine & Sulfadiazine for 3-6 weeks.

Cryptosporidiasis :

It is more prevalent in poorly developed countries and common in HIV when CD4 count goes below 50 cells/ml. It is caused by a protozoa Cryptosporidium parvum, tranmitted by fecal-oral route. Cryptosporidiasis is characterised by diarrhoea which may range in severity from mild diarrhoea to cholera like watery diarrhoea accompanied by abdominal cramps, nausea, vomiting and anorexia. Diagnosis is based on the identification of the parasite in the stool by Modified acid fast staining (10, 20). Treatment is symptomatic and ART is must for restoration of immune status.

Cryptococcosis :

This is caused by Cryptococcus neoformans when CD4 falls below 50 cells/ml. Cryptococcosis presents as subacute meningitis or meningoencephalitis with fever, malaise, headache, neck stiffness, altered mental status, photophobia, lethargy and memory loss. Diagnosis is by detection of cryptococcal antigen in CSF. 1:8 titre is significant. If not treated, the condition is fatal. It is treated by Amphotericin B(0.7mg/kg) intravenously for 2 weeks followed by Fluconazole 400mg orally for 8 weeks. ART is essential. Immune Reconstitution Syndrome is common when ART is instituted after Cryptococcal infection(36).

Pneumocystis jiroveci Pneumonia :

This occurs typically when CD4 count falls below 200cells/ml and more common in USA than in other parts of the world. It presents with progressive dyspnoea, dry cough, mild fever and weight loss. Diagnosis is by Chest Radiography where an interstitial infiltrate from perihilar to peripheral region is seen. Fibre optic bronchoscopy with bronchoalveolar lavage or transbronchial lung biopsy is done to demonstrate the morphology of the organism.

Trimethoprim 15mg/kg/day and Sulfmethoxazole 75mg/kg/day is given in three divided doses for 21 days. Corticosteroids may be added in case of moderate to severe disease. However it is ideal to start TMP-SMX regimen prophylactically when CD4 falls to 200 cell/ml.

Cytomegalovirus Infections :

Retinitis is the most common manifestation of CMV infection which typically occurs when CD4 falls below 50 cells/ml. It causes progressive necrotising retinitis first in one eye then affects the other eye also. The patients complain of floaters, blurring of vision and finally blindness. The virus also affects gastro intestinal tract causing esophageal, gastric, duodenal ulcers and entero-colitis. In the CNS, it causes CMV polyradiculopathy and CMV ventriculoencephalitis. In the lungs, pneumonitis is common. Treatment is i.v. ganciclovir followed by oral valganciclovir.

Mycobacterium Avium Complex :

MAC infection is common when CD4 count goes below 100 cells/ml. It is acquired through ingestion or inhalation. In HIV infection, involvement of gut is common leading to nausea, vomiting, watery diarrhoea. Dissemination is more common in MAC infections characterised by fever, night sweats and weight loss. Blood culture is diagnostic. Clarithromycin/azithromycin + Ethambutol + Rifabutin is the treatment usually given for life.

Bacterial Pneumonia :

It is caused by S.pneumoniae, H.influenzae, M.catarrhalis, K.pneumoniae, S.aureus. In addition Chlamydiae, Nocardiae, Legionellae, Rhodococcus equi are also associated. The patient

experiences fever, cough and pleuritic chest pain. Blood cultures are more diagnostic than sputum cultures. Treatment is Vancomycin 1g i.v. or Ciprofloxacin 750 mg orally.

Skin Infections:

These include impetigo, folliculitis, cutaneous abscesses, subcutaneous and soft tissue abscesses. Cauasative organisms are Staphylococcal and Streptococcal sp. Treatment is surgical drainage and appropriate antibiotics.

Bacillary angiomatosis is characterised by erythematous nodules caused by Bartonella henselae, B.quintana. Treatment is Erythromycin 500mg 4 times a day or Doxycycline 100mg bd for 1-2 months.

Enterocolitis :

Bacteria responsible for causing enterocolitis in HIV are non-typhoid Salmonella(S.typhimurium), Shigella, Campylobacter jejuni, Clostridium difficile. The manifestations include severe diarrhoea, abdominal cramps, nausea, fever. Treatment is Ciprofloxacin 500mg bd for 2-4 weeks. For C.difficile Vancomycin or Metronidazole is the drug of choice.

Progressive Multifocal Leucoencephalopathy :

This is a demyelinating illness caused by JC virus. The virus causes lysis of oligodendrocytes and breakdown of myelin producing white lesions in the brain. Diagnosis is by brain biopsy or PCR of CSF. There is no antiviral treatment but ART should be given.

Neoplasms :

Neoplasms common in HIV infection are Kaposi's sarcoma and Non Hodgkin's lymphoma followed by Multiple myeloma, melanoma, cervical, skin, oral and lung cancers.

Kaposi's Sarcoma :

It is characterised by many reddish purple nodules in skin, mucous membrane and viscera. The lesions vary in size from a few millimeters to several centimeters and are common in sun exposed areas and in areas of trauma. Skin, lymphnode, lungs and gastro intestinal tract are commonly affected followed by heart and CNS. Diagnosis is by biopsy of the histology showing extravasation of RBCs and proliferation of spindle and endothelial cells. Treatment is indicated only when the lesion overlies a joint or when it causes discomfort, cosmetic problems, difficulty in swallowing & breathing. It is treated by localised radiotherapy and intralesional Vinblastine. In other cases ART is enough.

Lymphomas :

These occur when CD4 count goes below 200 cells/ml. Three types of lymphoma are common in HIV – immunoblastomas, Burkitt's lymphoma, Primary CNS Lymphoma. Immunoblastomas account for 60% of the cases presenting as ascites, pleural or pericardial effusion without lymphadenopathy. Burkitt's lymphoma contributes 20% of cases and Primary CNS Lymphoma another 20%. The clinical features of lymphoma in HIV ranges from fever, night sweats, weight loss to focal neurological deficits.

IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME (IRIS) :

After starting ART, instead of clinical well being there is deterioration of the patient's health condition and worsening of Opportunistic infections. This usually occurs 2 weeks – 2 years of starting ART and is most common in patients in whom ART is started when CD4 count is under 50 cells/ml.

IRIS may be due to an untreated mycobacterial infection in the patient. The pathogenesis of IRIS may be due to a mechanism simulating Type IV hypersensitivity reaction. It may cause localised lymphadenopathy, fever, infiltrates in the lung, increased intracranial pressure, Grave's disease and uveitis. IRIS may end fatal. In severe cases, glucocorticosteroids and other immunosuppressive drugs are given (31).

LABORATORY DIAGNOSIS OF HIV (2, 31, 51) :

By early 1985, antibody based tests were developed in USA, by 1996 p24 antigen capture assay was developed and by 2002, nucleic acid testing was developed. The diagnosis of HIV depends on detection of HIV antibodies and direct demonstration of HIV or one of its components. Antibodies to HIV start to appear from 2-12 weeks of infection (31).

Specimen Collection & Transport :

For HIV-1 & 2 antibody testing and p24 antigen testing, about 5ml of clotted blood is sufficient. The samples must be properly labeled. If any delay in transport is anticipated, the Samples can be stored at room temperature for 3 days at 4°C. For viral RNA assays, 10ml of whole blood in EDTA tubes or 5ml of plasma is sufficient and can be stored at 2-25°C and transported to the laboratory within 48 hours as the viral DNA starts to denature.

The HIV test should be done for

1. Patients with TB, especially young
2. Patients with Sexually Transmitted Diseases (STD)
3. Antenatal care patients
4. Patients with Hepatitis B,C
5. History of high risk behaviour / transfusion.

DIAGNOSTIC TESTS :

Antibody based tests :

Screening tests

1. ELISA
2. Rapid tests

Supplemental tests

1. Immunofluorescent assay
2. Western blot
3. Line Immuno assay
4. Radio Immuno Precipitation Assay

Other tests :

1. P24 antigen tests
2. Polymerase Chain Reaction (PCR)
3. Plasma /serum viral load

Alternative tests :

1. Saliva HIV tests
2. Urine HIV tests

ELISA :

This is the widely used sensitive test for HIV infection because of its high sensitivity. The antigen is coated on microtitre wells. The test serum is added. If antibodies are present, it binds to the antigen. After washing, anti human immunoglobulin linked to a suitable enzyme is added followed by a colour forming substrate. If the test serum contains anti HIV antibodies, a colour is formed which can be detected visually. There are four generations of ELISA.

1. First generation – whole viral lysate
2. Second generation – Recombinant antigen
3. Third generation – synthetic peptide
4. Fourth generation – antibody + p24 antigen (HIV duo)

Using first and second generation ELISA, antibodies can be detected in 6-12 weeks. With third generation ELISA, antibodies can be detected in 3 weeks. With fourth generation ELISA since it detects p24antigen also HIV can be diagnosed in 2 weeks. But ELISA has the disadvantage producing false positive and false negative results.

RAPID TESTS :

These tests yield results within 30 minutes. When performed correctly, they are accurate. The results can be read by naked eye. These tests are useful in emergency room, autopsy room and smaller blood banks.

Advantages :

Rapid tests are useful in

1. Labour room
2. Mass screening.
3. Detecting HIV-2 also.

Disadvantages :

1. Subjective interpretation
2. A colour blind cannot interpret.

WESTERN BLOT :

This assay is based on the fact that various HIV antigens of different molecular weight induces the production of specific antibodies. The antibodies to each component produces a band. So the HIV proteins are separated according to their molecular weight and electrophoretic mobility by polyacrylamide gel electrophoresis and blotted on strips of nitrocellulose paper. The strips are reacted with test sera and then with anti human immunoglobulin conjugated with enzyme. A suitable substrate is then added which produces a colour band where specific antibody has reacted with the separated viral antigen. The position of the band indicates the antigen with which the antibody has reacted.

Interpretation :

Positive Western Blot Criteria :

1. WHO – 2 env with/without gag/pol
2. CDC – Any two p24, gp41, gp120, gp160

Negative – no bands

Indeterminate – bands present but does not satisfy the criteria.

P24 ANTIGEN TEST :

P24 antigen tests are also EIA based and use antibodies to capture the disrupted antigen. This antigen appears as early as two weeks and lasts for 3-5 month and so this test shortens the window period.

This test is useful :

1. During the window period
2. To detect HIV infection in newborn
3. During late disease when the patient is symptomatic.

Disadvantages :

1. Low sensitivity
2. Repeat tests needed for confirmation

PCR :

In this the target HIV RNA or proviral DNA is amplified enzymatically invitro by chemical methods. It is extremely sensitive as a single copy of DNA can be amplified. HIV nucleic acid is detectable as early as 12 days. Three different techniques are available RT-PCR, Nucleic Acid Sequence Based Amplification(NASBA) and branched DNA Assay.

HIV RNA measurement by PCR requires initial conversion of viral RNA into cDNA by addition of exogenous Reverse Transcriptase followed by PCR amplification and quantification

of the product. The commercially available RNA detection kits have a sensitivity of 40-80 copies/ml plasma.

Uses :

1. In hypogammaglobulinemia
2. In advanced disease
3. Drug resistance in HIV
4. Genotyping & Sequence analysis.

HIV DIAGNOSIS IN NEWBORNS :

Serologic diagnosis of HIV in newborn is difficult because anti HIV antibody coming from the mother persist for 18 months. If DNA PCR is positive within first 2 days, it is diagnostic of infection in utero. In infection during delivery, PCR is negative at 48 hours and positive within one month. In case of p24 antigen false positives are common in neonates. So it is inferior to PCR. For diagnosis in newborns, PCR should be done at 48 hours, 1 week, 3 months and six months and it should be confirmed by serology at 18 months.

NEWER TESTS :

ORASURE- SALIVA HIV TEST :

Non-invasively collected specimens like oral fluid, saliva and oral mucosal transudate are used. These systems detect antibodies comparable to or exceeding serum samples.

URINE TESTS :

IgG antibodies are found in urine. The collection of urine is simple, noninvasive and so more useful in developing countries where trained technicians are not available for collecting blood.

LAB MONITORING OF PATIENTS WITH HIV INFECTION :

CD4 COUNT :

CD4 count measures the degree of immunosuppression. CD4 progressively declines as the immune function decreases. It is used in staging the disease, monitoring disease progression, serves as a guide to start ART, determining treatment failure. CD4 cell count is the best predictor of disease progression and it is cheaper than viral load. So it is useful in developing and poor nations (66).

The WHO 2010 recommendations states that all HIV positive adults with CD4 count less than 350 cells/ml should be started on ART with or without symptoms. WHO defines immunological failure as fall of CD4 count to baseline or below or 50% decrease of CD4 count from on treatment peak value or persistent CD4 count below 100 cells/ml.

The U.S. CDC uses CD4 count and divides AIDS into three categories A, B, C. Category A CD4 count >500 cells/ml, Category B CD4 count 200-499 cells/ml, Category C CD4 count <200 cells/ml. According to CDC, AIDS is HIV infection with CD4 count < 200 cells/ml or CD4% < 14%. But WHO staging does not include CD4 count to accommodate poor nations (45).

HIV VIRAL LOAD :

It is a direct measure of in vivo replication of virus and therefore it is a powerful prognostic tool (26). Measurement of viral load is based on getting the target RNA, Reverse Transcription of RNA into cDNA, PCR amplification of target DNA and detection of dual fluorescent labeled oligonucleotide probes which quantifies HIV-1 target RNA. The test can detect and quantitate HIV-1 RNA as few as 40-50 copies/ml of plasma. Therapy is considered in patients with more than 1,00,000 copies/ml. During therapy, viral load is determined every 3-4 months. Reduction of viral load less than 50 copies/ml in 6 months indicates effective treatment.

Uses :

1. Guide for initiating ART
2. Optimising the duration of treatment.
3. Switching to second line of treatment
4. Diagnosing HIV infections in children < 18 months.

HIV DRUG RESISTANCE TESTING (31, 67) :

It measures the sensitivity of individual's HIV to different ARV agents which can be measured by phenotypic and genotypic methods. In genotypic assays, sequence analysis of patient's HIV is compared with sequence of virus with known ARV resistance profiles. In phenotypic methods the in vivo growth of the virus is compared with reference strains in the presence and absence of ARV agents.

Uses :

1. Selecting an initial regimen for treatment of new patients

2. Selecting new drugs in drug failure

TREATMENT :

The treatment of HIV includes counseling to the patient and ART.

Counseling :

1. The first and foremost is the emotional support to the patient to overcome the psychological blow.
2. Up – to date information about the disease, its severity should be given to the patient.
3. The need to start ART, its benefits, side effects should be informed.

Anti Retro Viral Therapy (31, 81) :

The main aim of ART is to suppress viral load so that the quality of life of the patient can be improved and the duration of life can be prolonged (18). If the HAART regimens are strictly followed, many opportunistic infections can be prevented.

Classification of drugs :

1. Drugs inhibiting the viral reverse transcriptase enzymes
    a. Nucleoside Reverse transcriptase inhibitors- Zidovudine, didanosine, Zalcitabine, Stavudine, Lamivudine, abacavir.
    b. Non –Nucleoside Reverse transcriptase inhibitors – Nevirapine, Delaviridine, Efavirenz.
    c. Nucleotide Reverse transcriptase inhibitors – Tenofovir.
2. Drugs inhibiting Protease enzyme – Ritonavir, Indinavir, Saquinavir, Amprenavir, Lopinavir, Nelfinavir,Atazanavir, Darunavir.

3. Drugs inhibiting Integrase enzyme – Raltegravir
4. Drugs inhibiting Viral entry – Maroviroc
5. Drugs inhibiting Fusion of viral envelope with host cell- Enfuvirtide.

Nucleoside Reverse transcriptase inhibitors :

These drugs enter the host cell and are phosphorylated into their respective triphosphate forms, inhibit the Reverse Transcriptase by competing with our host nucleosides and cause the chain termination of viral DNA.

DRUG	DOSAGE	TOXICITY
Zidovudine	300 mg bd	Anaemia, Neutropenia, myopathy, lactic acidosis, hepatomegaly
Didanosine	200mg bd 1 hour before food.	Pancreatits, Peripheral neuropathy, lactic acidosis, hepatomegaly.
Zalcitabine	0.75 mg tds	Pancreatits, Peripheral neuropathy, lactic acidosis, hepatomegaly, oral ulcers.
Stavudine	40 mg bd	Pancreatits, Peripheral neuropathy, lactic acidosis, hepatomegaly, lipodystrophy.
Lamivudine	150 mg bd	Hepatotoxicity
Emtricitabine	200 mg qd	Hepatotoxicity
Abacavir	300 mg bd	Fatal hypersensitivity, fever, rash

Non- Nucleside Reverse Transcriptase Inhibitors :

These drugs need not undergo intracellular phosphorylation and directly act on the RT enzyme at a different site and inhibit it. These are non-competetive inhibitors. They are not active against HIV-2. These drugs when used alone develop resistance quickly so always used in combination with other drugs.

DRUG	DOSAGE	TOXICITY
Delavirdine	400 mg tds	Skin rashes, Hepatotoxicity
Nevirapine	200 mg/day for 14 days then 200 mg bd	Skin rashes, Hepatotoxicity
Efavirenz	600 mg od before food	Skin rashes, Hepatotoxicity, depression, abnormal dreams.

Nucleotide Inhibitors :

Tenofovir is a newer drug and is the only nucleotide analogue. It is used in a dose of 300 mg qd. It is most effective and lest toxic. It is used when Zidovudine or Nevirapine cannot be used due to contraindications. However it can cause nephrotoxicity on prolonged use.

Protease Inhibitors :

Protease cleaves the large viral polyproteins into structural proteins and enzymes. These drugs inhibit the cleaving function of protease. These are more effective than NRTIs. As these drugs act on late stage of HIV replication cycle, they act on both newly and chronically infected cells.

DRUG	DOSAGE	TOXICITY
Saquinavir	1000mg+100mg ritonavir bd	Diarrhoea, Nausea, Abdominal pain, Head ache, fat redistribution, Lipid abnormality
Ritonavir	600mg bd	Nausea, Abdominal pain, Head ache, fat redistribution, Lipid abnormality
Indinavir	800mg+100mg ritonavir bd	Nephrolithiasis Indirect hyperbilirubinemia fat redistribution, Lipid abnormality.

Nelfinavir	1250mg bd	Nausea,Diarrhoea, hyperglycemia fat redistribution, Lipid abnormality
Amprenavir	600mg+100mg Ritonavir bd	Nausea, Abdominal pain, Head ache, fat redistribution,Lipidabnormality hyperglycemia
Lopinavir	100mg bd	Nausea, Abdominal pain, Head ache, fat redistribution,Lipidabnormality,hyperglycemia
Atazanavir	400mg qd	Hyperbilirubinemia, nausea, PR prolongation, hyperglycemia, fat redistribution
Tipranavir	500mg+200mg Ritonavir bd	Diarrhoea,fatigue, skin rash, headache, Hepatotoxicity,Intracranial haemorrhage.
Darunavir	600mg+100mg Ritonavir bd	Diarrhoea, nausea, headache

Integrase Inhibitors :

Integrase enzyme cuts the host DNA and integrates the viral DNA with it. Raltegravir inhibits this enzyme. There is no cross resistance with other ARV drugs and it is used against both HIV-1 & HIV-2. It is given in a dose of 400 mg bd. When used in combination with other drugs, it quickly produces an increment in CD4 count and decrement in HIV RNA levels. Side effects are nausea, rash.

Fusion Inhibitors :

Enfuvirtide binds with gp41 inhibits the fusion of viral envelope with the plasma membrane of host cells thus the viral entry into the host cells is prevented. It is not active against HIV-2. No cross resistance with other drugs is observed. It is used in patients who failed to recover with previous regimens. Dosage is 90mg bd subcutaneously. Disadvantages are it is costly, painful causing nodules at injection sites.

Entry Inhibitors :

Maroviroc is a new drug that blocks the CCR5 receptor of host cells and the attachment and viral entry into the host cells are inhibited. So useful only against R5 viruses and not active against X4 viruses which use CXCR4 receptor. Receptor tropism assays are performed before using it. It is used in patients with multiple drug resistant CCR5 tropic HIV infections. Dosage is 150-600 mg bd orally. Side effects are hepatotoxicity, fever, rash, abdominal pain, immunosuppression as it involves the human chemokine receptor.

Therapeutic Regimen :

1. ART when started, it should be with 3 drugs belonging to 2 different classes.
2. For patients who are started on ART, Protease Inhibitors are not used. PIs(Low dose Ritonavir boosted PIs) are used for patients who failed earlier regimens.
3. In cases of side effects, the causative drug or the entire regimen may be changed. In case of treatment failure the entire regimen is changed.
4. Treatment is life-long.
5. In pregnancy, the drugs like Zidovudine, Lamivudine, Nevirapine, Nelfinavir and Saquinavir are relatively safe.
6. The universally preferred first line regimen is 2NRTIs + 1NNRTI. The preferred

NACO RECOMMENDED FIRST LINE REGIMEN :

1.	PreferredRegimen	Lamivudine + Zidovudine + Nevirapine
2.	Alternative Regimen	Lamivudine + Zidovudine + Nevirapine Lamivudine + Stavudine + Efavirenz Lamivudine + Stavudine + Nevirapine
3.	Other options	Lamivudine + Tenofovir + Nevirapine Lamivudine + Tenofovir + Efavirenz Lamivudine + Zidovudine + Tenofovir

If the patient is anaemic, Stavudine is used instead of Zidovudine.

When Zidovudine & Stavudine are contraindicated, Tenofovir is used.

Efavirenz is used in those with liver dysfuntion and those using Rifampicin.

Nevirapine is used in pregnant women.

When Efavirenz & Nevirapine are contraindicated, 3 NRTIs are used (2).

Indications for Changing Regimen :

1. When plasma RNA drops less than a log or increases significantly after 4 weeks of initiation of therapy.
2. Decreasing CD4 counts
3. Clinical deterioration
4. Drug Toxicities

Second line Regimen :

Drug susceptibility testing is done for choosing the optimal regimen. It is measured by phenotypic or genotyping methods. Phenotypic assays measure the activity of viral enzymes in the presence or absence of different concentrations of drugs. Genotyping assays done by DNA chip hybridisation & line probe assays. A Ritonavir boosted PI is included. Maroviroc, Enfuvirtide, Raltegravir may be considered in cases of repeated failures.

SECOND LINE REGIMENS :

NRTI	PI
1. Tenofovir + Abacavir	Lopinavir/r
2. Didanosine + Abacavir	Atazanavir/r
3. Tenofovir + Zidovudine	Saquinavir/r
4. Tenofovir + Lamivudine	Indinavir/r

POST – EXPOSURE PROPHYLAXIS :

Health care personnel who are accidently exposed to the risk of HIV are considered for PEP. The goal of PEP is to suppress viral replication so that wide spread dissemination and infection is prevented.

Low risk	Exposure through mucous membrane, superficial scratch, thin needle when the source is asymptomatic with high CD4 & low RNA	Zidovudine 300mg + Lamivudine 150mg bd for 4 weeks
High risk	Exposure through large splash, large area of mucous membrane, abraded skin, large bore needle when the source is symptomatic with high RNA & low CD4	Zidovudine 300mg bd + Lamivudine 150mg bd + Indinavir 800 mg tds for 4 weeks

Perinatal Prophylaxis :

NACO first line regimen for HIV positive pregnant women is Zidovudine + Lamivudine + Nevirapine. For those who are not on ART, Zidovudine 300mg bd is started during the second trimester and continued through delivery, postnatal period and the neonate is treated for 6 weeks

DRUG RESISTANCE :

Resistance is common when monotherapy instead of combination therapy is given and when the replication rate of virus is high. It is due to mutations of the aminoacids of viral enzymes. The resistance to NRTIs may be due to a mutation in the Reverse Transcriptase enzyme that identifies the drug and prevents the attachment of the drug to the primer. The other mechanism is mutation in the enzyme that increases the removal of NRTIs. A mutation in the hydrophobic pocket of the Reverse Transcriptase enzyme prevents the binding of NNRTIs .A mutation in the protease enzyme produces structural changes in the enzyme so the affinity between drug and enzyme is reduced. Drug resistance is monitored by CD4 counts, HIV RNA levels and PCR.

VACCINES (31, 67) :

There is not a safe, effective vaccine for HIV till now because of the following reasons :

1. The immune response does not clear HIV infection in turn causes superinfection. This also holds good for the vaccine immunity.
2. High mutation rate of virus due to error prone nature of Reverse Transcriptase.
3. Different strains of HIV are prevalent worldwide. There is no international cooperation to produce a candidate vaccine that is suitable for the strains prevalent in developed and developing countries.
4. The vaccines are greatly needed in developing countries where the people are poor and have the least ability to pay.

MATERIALS & METHODS

STUDY PLACE :

This study was conducted among cases attending Voluntary Counseling Testing Centre, Thanjavur Medical College Hospital.

STUDY PERIOD :

The study period was 1 year from September 2012 to September 2013.

STUDY POPULATION :

The study population includes 346 HIV positive patients detected by antibody test.

STUDY DESIGN :

Observational study.

ETHICAL CONSIDERATIONS :

Written consent to participate in the study was obtained from the patients or their guardians after full explanation of the study. This study was reviewed by Institutional Ethical Committee, Thanjavur Medical College, Thanjavur. All data were handled confidentially and anonymously.

INCLUSION CRITERIA :

Cases attending VCTC , TMCH, Thanjavur like

1. Cases referred from the Surgical specialities for HIV testing as a part of routine screening.

2. Cases referred from Medical wards with history suggestive of HIV.
3. Cases referred from ENT and dental OPD with signs of oral thrush.
4. Cases referred from skin OPD with herpes like lesions.
5. Cases referred from STD clinics with genito urinary lesions.
6. All Cases with sputum smear positivity referred from TB cell .
7. Spouses of HIV reactive cases.

EXCLUSION CRITERIA :

1. Antenatal mothers.
2. Neonates born to HIV positive mothers.
3. Patients on Anti Retro Viral therapy.
4. Cases of chronic diarrhea not responding to routine antibiotics.

Clinical data were collected from all patients.

SAMPLE COLLECTION :

After giving pretest counseling and obtaining written consent from the patient, 5ml of blood was collected from the patients by intravenous route with universal precautions and transferred to sterile screw capped vials. The caps were fixed with adhesive tape to prevent leakage during transport. The blood samples were transported to the lab immediately in an ice box with proper labeling (name of the patient, identification number, date of collection)

SAMPLE PROCESSING :

The samples were taken to the laboratory immediately and they were allowed to clot by placing in a rack at room temperature (20 - 25°C) for at least 30 minutes. After that serum was separated by centrifugation. The clarified serum was then transferred to a sterile vial and was stored at +4°C (short term storage) and at -70°C (long term storage).

METHODS :

- All cases were screened by COMB-AIDS kit at VCTC, TMCH, Thanjavur.
- Those samples which test reactive to COMB-AIDS kit are subjected to PAREEKSHAK HIV 1/2 TRILINE CARD test at VCTC, TMCH, Thanjavur.
- Those samples which test reactive to the second test are subjected to AIDSCAN HIV 1/2 TRISPOT TEST KIT procedure at VCTC, TMCH, Thanjavur.
- The samples are further confirmed by HIV ELISA.
- CD4 counts of the reactive patients were detected by BD-FACS counter at ART centre, TMCH, Thanjavur.
- The sputum of the patients with symptoms of cough, fever and weight loss were screened for Pulmonary Tuberculosis by Zeihl – Neelsen staining.
- Oral swabs were taken from those with oral thrush and streaked on SDA.
- All the samples were screened for Toxoplasmosis by Toxoplasma IgG ELISA.
- All the samples were screened for HSV-2 by HSV-2 IgG ELISA.
- All the samples were screened for Cryptococcosis by Latex Agglutination Test.
- AMPLICOR HIV-1 DNA PCR, version 1.5was run for 10 samples to detect HIV-1 DNA.

COMB – AIDS – RS ADVANTAGE – ST:

This test kit is an in-vitro, visually read dot immunoassay for qualitative detection of antibodies to HIV in blood, serum or plasma.

Principle :

It employs the principle of dot immunoassay where the immobilized antigen – antibody complex is visualized by a colored end point produced by Colloidal gold – Protein A signal reagent. Each tooth of the comb has two circular spots, one near the tip of the comb blended with synthetic peptides or recombinant antigens of HIV(Test spot) and the other spot little above the first spot blended with the control reagent (Control spot). A positive test result is indicated by a pink spot/dot in the test spot near the tip of the comb.

REAGENTS/ACCESSORIES IN THE KIT :

Reagents :

1. Washing buffer
2. Colloidal gold signal reagent
3. Sample diluent
4. Positive control
5. Negative control
6. Antigen & control coated combs

ASSAY PROCEDURE :

1. All kit components and samples are brought to room temperature.
2. Add 2 drops of sample diluent to each micro test wells.
3. Add 2 drops of sample to each of the above well.
4. Place the required number of combs into respective wells.
5. Incubate for 10 minutes at room temperature.
6. Wash the combs in buffer solution
7. Add 4 drops of colloidal gold reagent in another micro test wells.
8. Place the combs in micro test wells containing colloidal gold signal reagent.
9. Incubate for 10 minutes at room temperature.
10. Wash the combs in buffer solution.

INTERPRETATION :

Positive Result :

This is indicated by the presence of pink colored spot or dot both in the test and control spot.

Negative result :

This is indicated by the presence of pink colored spot or dot in the control spot alone.

Indeterminate result :

This is indicated by the presence of faint pink colored spot or dot both in the test and control spot. In this case, the test should be repeated. If the result is still indeterminate, fresh sample is taken after 4-8 weeks and tested again.

Invalid result :

This is indicated by the absence of pink coloured spot or dot in the control area irrespective of presence or absence of pink coloured spot or dot in test area.

PAREEKSHAK HIV 1/2 TRILINE CARD TEST :

It is an immuno chromatography based assay for detection of antibodies to HIV-1 and HIV-2.

Principle :

This test follows lateral flow immuno- chromatography. The test device has a sample window with a reagent releasing pad which is held in contact by a porous membrane material. The membrane has four zones. The first zone is mobile and it is at the sample window and it consists of gold particles sensitised with HIV antigens. The second and third zones(Test line) has recombinant HIV antigens(recombinant HIV gp-41antigen and C terminal of gp120 for HIV-1& recombinant HIV-2 gp36 for HIV-2) immobilised on the membrane. The fourth zone has(control line) has control antibody immobilised on the membrane.

If the sample has antibodies they form a complex with HIV antigen conjugated gold, move on trapped by test line and a red line is formed. The unbound colloidal gold particles continue to move due to capillary action, come in contact with the control line, trapped giving rise to a red line demonstrating the validity of the test.

CONTENTS OF THE KIT :

1. Testing device
2. Diluent
3. 10 µl dropper
4. Silica gel
5. HIV – 1&2 positive control
6. HIV – 1&2 negative control

ASSAY PROCEDURE :

1. Bring the kit and samples to room temperature.
2. Remove the test device from the pouch just before the test.
3. Place the device on a flat surface.
4. Add a 1drop of serum or plasma into the sample window and allow it to soak in.
5. Add 2 drops of diluent into the sample window.
6. Read the results in 20 minutes.

INTERPRETATION OF RESULTS :

Negative result :

The presence of only one band at ‘C’ indicates negative result.

Positive result :

The presence of a band at ‘C’ and bands at ‘1’ and /or at ‘2’ indicates positive result for HIV-1 or HIV-2.

Invalid result :

If the control band is not visible and only the test band is visible, then the test is invalid.

HIV ELISA MICROLISA :

This is developed to detect anti-HIV envelope antibodies to HIV-1 and HIV-2 with equal reactivity. The core proteins show cross reactivity and envelope proteins are found in all persons. So in this test antibodies to envelope proteins like gp41, C terminus of gp120 and gp36 are detected.

Principle :

HIV envelope proteins are coated on the surface of microtitre wells. If the patient's sample has anti-HIV antibodies, it will bind to the antigen. After washing to remove the unbound material, anti human immunoglobulin conjugated with HRP is added which binds only to the antigen – antibody complex. Then the substrate containing chromogen and hydrogen peroxide is added and incubated. A blue colour develops in proportion to the amount of HIV-1 or HIV-2 antibodies. The reaction is read at a wavelength of 450 nm. If the sample does not contain antibody then the conjugate will not bind and no colour develops.

COMPONENTS IN THE KIT :

1. Microwells coated with HIV-1 & HIV-2 recombinant proteins. (12x8 wells)
2. Sample diluent 20ml.
3. Anti – human immunoglobulin coated with Horse Radish Peroxidase – 0.25ml
4. Conjugate diluent 15 ml

5. Wash buffer 50ml
6. TMB substrate 10ml
7. TMB diluent 10 ml
8. Positive control 2ml – inactivated diluted patient's serum positive for HIV antibodies.
9. Negative control 2 ml – normal serum negative for HIV, HbsAg, HCV.
10. Stop solution – 15 ml
11. Plate sealers.

TEST PROCEDURE :

1. Add 100µl sample diluent to A-1 as blank.
2. Add 100µl Negative control to B-1 & C-1 wells.
3. Add 100µl Positive control to D-1, E-1& F-1 wells.
4. Add 100µl sample diluent in each well starting from G-1followed by addition of 10µl sample.
5. Apply cover seal.
6. Incubate at 37°C for 30 minutes.
7. Wash the wells 5 times.
8. Add 100µl of working conjugate in each well from A-1.
9. Apply cover seal.
10. Incubate at 37°C for 30 minutes.
11. Add 100µl working substrate in each well from A-1.
12. Incubate at room temperature for 30 minutes in dark.
13. Add 100µl stop solution.
14. Read absorbance at 450 nm within 30 minutes in ELISA reader.

INTERPRETATION OF RESULTS:

1. Cut-off value is calculated is by adding mean negative control and mean positive control and the sum is divided by 6.
2. Test specimens with absorbance less than the cut-off value are non reactive and negative for anti-HIV.
3. Test specimens with absorbance equal to or greater than the cut-off value are reactive for anti-HIV.
4. This is a screening test and should be confirmed by Western blot as false positive results are caused by Leprosy, TB, Herpes simplex virus, lipemic serum.

CD4 DETERMINATION BY BD-FACS COUNT SYSTEM

The BD-FACS Count System for use with the BD FACS Count CD4/CD3 reagent kit is an automated instrument designed for enumerating the absolute cell counts of CD4 and CD3 T lymphocytes in unlysed whole blood.

Principle :

When whole blood is added to the reagents, fluorochrome – labeled antibodies in the reagents bind specifically to lymphocyte surface antigens. When the sample is run on the instrument, the cells come in contact with the laser light which cause the fluorochrome – labeled cells to fluoresce(45). Analysis is automatic and the software calculates the absolute counts. Results are print immediately.

MATERIALS REQUIRED :

Materials provided :

1. Coring station – opens the reagent and control tubes to prepare them for use.
2. Electronic Pipette – delivers 50µl fluid.
3. Software protocol floppy discs
4. Workstation – holds blood samples and operating supplies.
5. System fluid – saline solution flowing through the fluidics
6. Thermal printer paper
7. Waste reservoir
8. Cleaning tubes and dispensing bottles.

PROCEDURE :

1. For each patient sample, label a reagent tube with patient accession number.
2. Vortex each tube upside down for 5 seconds.
3. Open each reagent tube with coring station.
4. Invert each BD vacutainer tube 5 to 10 times to adequately mix the blood.
5. Pipette 50µl whole blood into each reagent tube.
6. Cap each tube and vortex upright for 5 seconds.
7. Incubate the tubes for 60 to 120 minutes at room temperature
8. Pipette 50µl fixative solution into each one.
9. Recap each tube and vortex upright for 5 seconds.
10. Place the tube in the sample holder of the instrument.
11. Press run.

12. When the analysis is complete, the results are displayed.

ZEIHL – NEELSEN STAINING :

1. Primary staining with is done with strong Carbol fuschin for 5-7 minutes with intermittent heating.
2. Decolourising agent 20% Sulphuric acid is added and kept for 2 minutes.
3. Counter staining is by adding methylene blue and keeping it for 3 minutes.

Grading of the smear :

No. of bacilli	**Grade**
No bacilli in 300 fields	Negative
1 -2 bacilli/ 300 fields	Doubtful, Repeat the smear.
1-9 bacilli / 100 fields	1+
1-9 bacilli / 10 fields	2+
1-9 bacilli/field	3+
10 or more /field	4+

ORAL CANDIDIASIS :

1. Those patients with oral thrush were asked to rinse their mouth.
2. Two swabs were taken from the lesion.
3. One swab was used for Gram staining which shows budding gram positive cells with pseudohyphae.

4. The other was used to streak it on the Saburaoud's Dextrose Agar and Incubated at 37°C for 24 hours. Cream colored, smooth, pasty colonies appear. Gram staining from the colonies shows budding gram positive cells with pseudohyphae.

TOXOPLASMA IgG ELISA :

IgM & IgG antibodies to Toxoplasma can be detected with 2-3 weeks of exposure. IgG antibodies remains positive but the antibody level drops.

Principle :

Diluted serum is added to wells coated with purified Toxoplasma antigen. Toxoplasma IgG antibody if present, binds to the antigen. All unbound materials are washed away and enzyme conjugate is added which binds to antigen antibody complex, then substrate is added. The intensity of colour generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS IN THE KIT :

1. Microwells coated with Toxoplasma – 12X8 wells.
2. Sample diluent – 22ml
3. Calibrator – 1ml
4. Positive control – 1ml
5. Negative control – 1ml
6. Enzyme conjugate – 12ml
7. TMB substrate – 12ml
8. Stop solution – 12ml

9. Wash concentrate 20X – 25ml

ASSAY PROCEDURE :

1. Bring all specimens and reagents to room temperature.
2. Place the desired number of coated strips in the holder.
3. Prepare 1:21 dilution of the test samples by adding 10µl sample to 200µl diluent. Mix well.
4. Dispense 100µl diluted sera, calibrator and controls to appropriate wells.
5. Incubate for 20 minutes at room temperature.
6. Wash wells 3 times.
7. Dispense 100µl of enzyme conjugate in all wells.
8. Incubate for 20 minutes at room temperature.
9. W ash wells 3 times.
10. Dispense 100µl of TMB substrate in all wells.
11. Incubate for 10 minutes at room temperature.
12. Add 100µl stop solution.
13. Read the absorbance at 450 nm.

INTERPRETATION OF RESULTS :

Calculation :

1. Cut-off value = Calibrator OD X Calibrator factor
2. Antibody index is calculated by dividing the OD value of the sample by cut-off value.
3. Antibody index < 0.9 – No detectable IgG antibody to Toxoplasma by ELISA.
4. Antibody index 0.9-1.1 : Borderline positive. Follow up needed if clinically indicated.

5. Antibody index > 1.1 – Detectable IgG antibody to Toxoplasma by ELISA.

HSV-2 IgG ELISA :

This is intended for the detection of IgG antibodies to HSV-2 in human serum or plasma. The presence of IgG is indicative of previous exposure. A significant increase in IgG is indicative of reactivation, current or recent infection.

Principle :

Diluted serum is added to wells coated with purified antigen. IgG specific antibody if present, binds to the antigen. All unbound materials are washed away and enzyme conjugate is added which binds to antigen antibody complex. Substrate is added and the plate is incubated to allow hydrolysis of substrate by enzyme. The intensity of colour generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS IN THE KIT :

1. Microwells coated with HSV-2 antigen – 12X8 wells.
2. Sample diluent – 22ml
3. Calibrator – 1ml
4. Positive control – 1ml
5. Negative control – 1ml
6. Enzyme conjugate – 12ml
7. TMB substrate – 12ml
8. Stop solution – 12ml
9. Wash concentrate 20X – 25ml

ASSAY PROCEDURE :

1. Bring all specimens and reagents to room temperature.
2. Place the desired number of coated strips in the holder.
3. Prepare 1:21 dilution of the test samples by adding 10µl sample to 200µl diluent. Mix well.
4. Dispense 100µl diluted sera, calibrator and controls to appropriate wells.
5. Incubate for 20 minutes at room temperature.
6. Wash wells 3 times.
7. Dispense 100µl of enzyme conjugate in all wells.
8. Incubate for 20 minutes at room temperature.
9. W ash wells 3 times.
10. Dispense 100µl of TMB substrate in all wells.
11. Incubate for 10 minutes at room temperature.
12. Add 100µl stop solution.
13. Read the absorbance at 450 nm.

INTERPRETATION OF RESULTS :

Calculation :

1. Cut-off value = Calibrator OD X Calibrator factor

2. Antibody index is calculated by dividing the OD value of the sample by cut-off value.

3. Antibody index < 0.9 – No detectable IgG antibody to HSV-2 by ELISA.

4. Antibody index 0.9-1.1 : Borderline positive. Follow up needed if clinically indicated.

5. Antibody index > 1.1 – Detectable IgG antibody to HSV-2 by ELISA.

CRYPTOCOCCAL LATEX AGGLUTINATION TEST :

Cryptococcal meningitis is a common opportunistic infection in AIDS. This test detects the Cryptococcal antigen in patient's serum or plasma.

Principle :

Latex particles coated with anticryptococcal globulin reacts with Cryptococcal Polysaccharide antigen causing visible agglutination.

MATERIALS IN THE KIT :

1. Latex particles coated with rabbit anti – cryptococcal globulin.
2. Negative control – normal human serum negative for HIV, HCV, HBsAg.
3. Positive control – Purified Cryptococcal neoformans polysaccharide.
4. Cards
5. Applicator stick.

PROCEDURE :

1. Remove the cards from the pack.
2. Label the cards as Negative control, Positive control and sample.
3. Add a drop of negative control, positive control and sample to designated rings.
4. Add a drop of latex to all the rings.
5. Rock the slide back and forth with hand or rotator at a speed of 125 rpm.
6. Read the agglutination titre.

INTERPRETATION :

a. Negative – homogenous with no clumping
b. +1 – Fine granulation against a milky background.
c. +2 – Small clumps against cloudy background.
d. +3 – Large & small clumps against clear background.
e. +4 - Large clumps against clear background.

AMPLICOR HIV-1 DNA PCR :

Sample Preparation :

HIV-1 DNA is isolated by washing the whole blood sample to extract the leucocytes which are then lysed in a detergent solution containing Proteinase K.

PCR Amplification :

This uses the primers SK145 and SKCC1B to define a sequence of 155 nucleotides within the highly conserved region of the HIV-1 gag gene. PCR amplification is performed with

thermostable recombinant enzyme Thermus thermophilus DNA polymerase in presence of manganese and appropriate buffer conditions.

Hybridization Reaction :

Following PCR amplification, the HIV-1 amplicon is denatured to form single stranded DNA by adding denaturing solution. Aliquots of denatured amplicon are added to separate wells of microtitre plates coated with HIV-1 specific oligonucleotide probe. The biotin labeled HIV-1 amplicon are hybridised to target specific oligonucleotide probes bound to wells of the MWP.

Detection Reaction :

Following the hybridisation reaction, the MWP is washed to remove unbound material and Avidin HRP Conjugate is added to each well of MWP. The Avidin HRP Conjugate binds to the biotin labeled HIV-1 amplicon hybridised to target specific oligonucleotide probes bound to wells of the MWP. The MWP is washed and substrate solution containing Hydrogen Peroxide and 3,3',5,5'- tetramethyl benzidine is added to the wells. In presence of Hydrogen Peroxide, the bound HRP catalyses the oxidation of TMB to form coloured complex. The reaction is stopped by addition of a weak acid and the absorbance at 450nm is measured.

REAGENTS :

Specimen Preparation Reagents:

1. Wash solution
2. HIV- 1 Extraction Reagent (Tris- KCl buffer, 0.1% detergent,0.01% Proteinase K)

Amplification Reagents:

1. HIV-1 master mix (Glycerol, <0.01% rTth polymerase, <0.07% dATP, dCTP, dGTP, dTTP, dUTP, <0.001% SK145 and SKCC1B biotinylated primers)
2. Manganese solution
3. HIV-1 positive control – non infectious DNA containing HIV-1 sequences
4. HIV-1 negative control – non infectious mammalian DNA.
5. HIV-1 internal control – non infectious plasmid DNA.
6. Avidin Horse Radish Peroxidase Conjugate
7. Substrate – TMB
8. Stop solution

Detection Reagents :

1. HIV-1 Micro Well Plate coated with HIV-1 specific DNA probe SK102.
2. HIV-1Internal Control Micro Well Plate coated with IC specific DNA probe CP35
3. Denaturation solution – 1.6% sodium hydroxide, EDTA, Thymol blue
4. HIV-1 Hybridisation buffer
5. Avidin – HRP
6. Substrate A solution – 0.01% Hydrogen Peroxide
7. Substrate B solution – 0.1% TMB
8. Stop solution – 4.9% sulphuric acid

PROCEDURE :

Reagent Preparation :

1. Place the reaction tubes in the MicroAmp tray and arrange in coloumns of 8 tubes each.

2. Prepare working Master mix by adding 100µl HIV-1 manganese solution to a vial of HIV-1 MMX. Recap the tube and invert it 10-15 times.
3. Add 50µl working Master mix into each reaction tube.

S ample and Control Preparation:

1. Add 1ml of BLD WS to 2ml screw cap tubes
2. Invert the tube of whole blood 10-15 times for mixing thoroughly.
3. Pipette 500µl whole blood into a tube containing BLD WS.
4. Incubate 5 minutes at room temperature.
5. Microcentrifuge the tubes for 3 minutes at maximum speed.
6. Aspirate the supernatant and add 1ml BLD WS to resuspend the pellet.
7. Repeat steps 5&6.
8. The dry pellet is extracted.
9. Working extraction reagent is prepared by adding 6ml HIV-EXT and 80µl HIV-IC and vortex the mixture for 10 seconds.
10. Add 200µl Working extraction reagent to each pellet, HIV-1 PC, HIV-1 NC and vortex.
11. Incubate the tubes at 60°C for 30 minutes in a dry sand block.
12. Incubate the tubes at 100°C for 30 minutes in a dry sand block.
13. Vortex the samples.
14. Microcentrifuge the samples for 3 seconds.
15. Remove MicroAmp trays from plastic bags.
16. Add 50µl of samples and controls to appropriate reaction tubes.

17. Move prepared samples and controls in the MicroAmp tray to the Amplification/Detection area.

Amplification :

1. Place the tray into thermal cycler block for 90 minutes.
2. Remove the tray from thermal cycler.
3. Add 100µl denaturation solution to the reaction tubes.
4. Incubate for 10 minutes at room temperature.

Detection :

1. Warm all reagents to room temperature.
2. Prepare working wash solution by adding 10X WB to 9 ml distilled water.
3. Allow HIV-1 MWP and HIV-1 CT MWP to warm to room temperature.
4. Add 100µl HIV-1 HYB to each well on the MWP.
5. Pipette 25µl of denatured amplicon to appropriate wells of the MWP. Gently tap it 10-15 times till the blue colour changes to yellow.
6. Incubate for 1 hour at 37°C.
7. Wash the MWP 5 times.
8. Add 100µl AV-HRP to each well.
9. Incubate at 37°C for 15 minutes.
10. Prepare working substrate by mixing 2ml of Substrate A and 0.5ml of Substrate B.
11. Wash the MWP 5 times.
12. Add 100µl working substrate to each well of MWP.
13. Incubate at room temperature for 10 minutes.

14. Add 100µl stop solution to each well of MWP.

15. Measure the absorbance at 450nm.

INTERPRETATION :

HIV result A450nm	IC result A450nm	Interpretation
< 0.2	>0.2	HIV -1 DNA not detected
<0.2	<0.2	Inhibitory sample. Should be repeated.
>0.8	ANY	HIV-1 DNA detected. Sample is positive for HIV-1 DNA.
>0.2, <0.8	ANY	Equivocal. Duplicate testing performed.

RESULTS

This study was carried out among 11953 cases attending VCTC of a tertiary care hospital, Thanjavur Medical College Hospital and over a period of one year.

- 11953 cases attending VCTC, TMCH, Thanjavur were analysed over a period of one year from September 2012 to September 2013.
- Of the 11953 cases, 346 cases were found to be reactive for HIV by Comb AIDS, HIV Triline and HIV Trispot.
- Among the 346 reactive cases, 346 cases were reactive for HIV-1, 1 case was reactive for both HIV-1 & HIV-2 and none were reactive for HIV-2 alone.
- All the 346 cases reactive to HIV by Comb AIDS, HIV Triline and HIV Trispot were also reactive by HIV Microlisa.
- The prevalence of HIV in this study over a period of one year was 2.8%.
- In this study, out of the 346 reactive cases 207(59.83%) were males and 139 (40.17%) were females.(Table 2)
- The male to female ratio was 1.48:1.
- The majority of the reactive cases, 146 (42.20%) out of 346 were in the age group of 31 to 40 years followed by 84 cases (24.28%) in the age group of 41 to 50 years.(Table 3)
- Among the reactive 8 (2.31%) were children.
- On the basis of habitat, people coming from rural areas (77.17%) outnumbered the urban population (22.83%) (Table 4)

- The education wise distribution showed that 113(32.66%) were illiterates 123(35.55%) were educated upto primary level, 68(19.65%) were educated upto secondary level and 35(10.12%) were graduates.(Table 5).
- Among the males, majority were farmers(35.27%) followed by laborers(33.33%) and drivers(17.40%).
- Among the females, majority were house wives(88.49%) .(Table 6)
- The distribution of the subjects showed that 312(90.17%) out of 346 were married and 21(6.07%) were unmarried. (Table 7)
- Heterosexual route (97.11%) was the major mode of transmission, 8 cases(2.31%) by perinatal transmission and 2 cases(0.58%) gave history of blood transfusion. (Table 8)
- According to the first CD4 count at the time of registration at the ART centre, the minimum CD4 count was 16 cells/ml and the maximum CD4 count was 1092 cells/ml with the mean CD4 count of 388.6 cells/ml.
- 90 of 346 cases (26.01%) had CD4 count > 500 cells/ml.
- 256 of 346 had CD4 count < 500 cells/ml, consisting of 80 (23.12%) with CD4 count between 350-500, 80(23.13%) with CD4 count between 201-350, 58 (16.76%) with CD4 count between 101 -200 and 23 (6.65%) with CD4 count between 51 – 100 and 15(4.34%) with CD4count < 50 cells/ml.(Table 8)
- The mean CD4 count of males is 389.67 and the mean CD4 count of females is 409 and the mean CD4 count of females is higher than that of males.
- The most common clinical presentation was fever(57%), weight loss (45%), oral thrush(35%), cough (15%) and primary generalised lymphadenopathy (10%).

- Among the HIV positive patients, 135 cases(39.02%) had oral thrush with mean CD4 count 409.97 and candida was grown when the swabs were streaked in SDA (Table 10, 11).
- Among the reactive cases, 97(28.03%) of them showed sputum smear positivity for Pulmonary Tuberculosis with the mean CD4 count 173.37 (Table 12, 13).
- Among the 346 sera screened for Herpes Simplex Virus -2 by IgG ELISA, 50 cases(14.45%) were reactive for HSV-2 with a mean CD4 count of 194.18 cells/ml (Table 14, 15).
- Among the 346 sera screened for Toxoplasmosis by IgG ELISA, 20 cases(5.78%) were reactive with a mean CD4 count of 182.35 cells/ml (Table 16, 17).
- Among the 346 sera screened for Cryptococcosis by Latex Agglutination test, 12 cases(3.41%) were reactive with a mean CD4 count of 96.58 cells/ml (Table 18, 19).
- So in my study the most common Opportunistic Infection among the HIV positive patients is Oral Candidiasis followed by Pulmonary Tuberculosis, Herpes Simplex Virus-2, Toxoplasmosis and Cryptococcosis.
- Ten whole blood samples with CD4 count less than 200 were subjected to HIV-1PCR and all the samples answered positive.

Table – 1. SEROPREVALENCE OF HIV

Total No. of cases tested	HIV Positive Cases	Prevalence (%)
11953	346	2.8%

Prevalence Rate = 346/11953 X 100 = 2.8%

Chart - 1

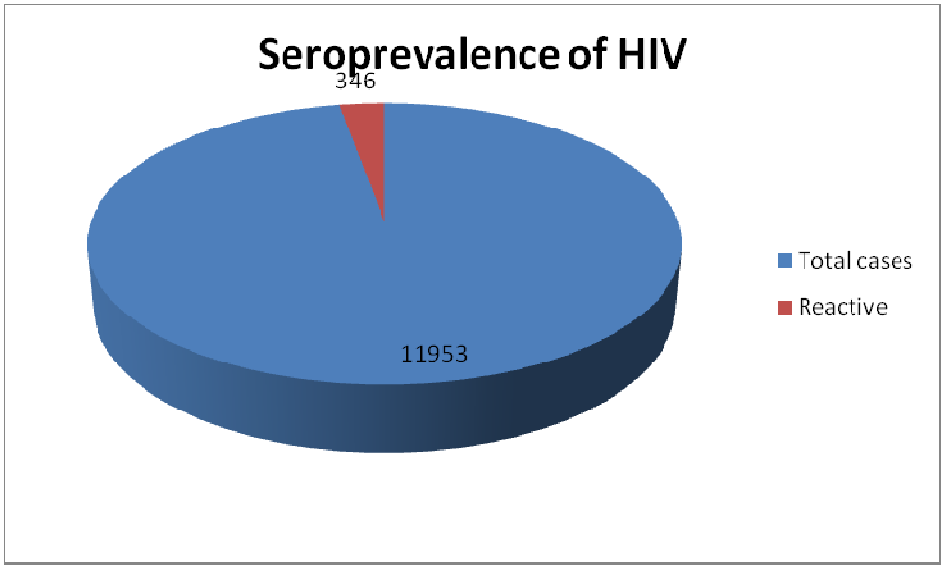


Table -2

SEX WISE DISTRIBUTION OF HIV CASES

S.NO	SEX	NUMBER	PERCENTAGE
1.	Male	207	59.83%
2.	Female	139	40.17%

Majority of the affected cases are males.

TABLE – 3

AGE WISE DISTRIBUTION OF HIV CASES

S.No	Age group	Total	Male	Female
1.	0-10 years	8	5	3
2.	11-20 years	7	5	2
3.	21-30 years	53	29	24
4.	31-40 years	146	73	73
5.	41-50 years	84	61	23
6.	51-60 years	41	29	12
7.	61-70 years	6	4	2
8.	> 70 years	1	1	0

Majority of the affected males and females are in the age group of 31-40 years.

Chart - 2

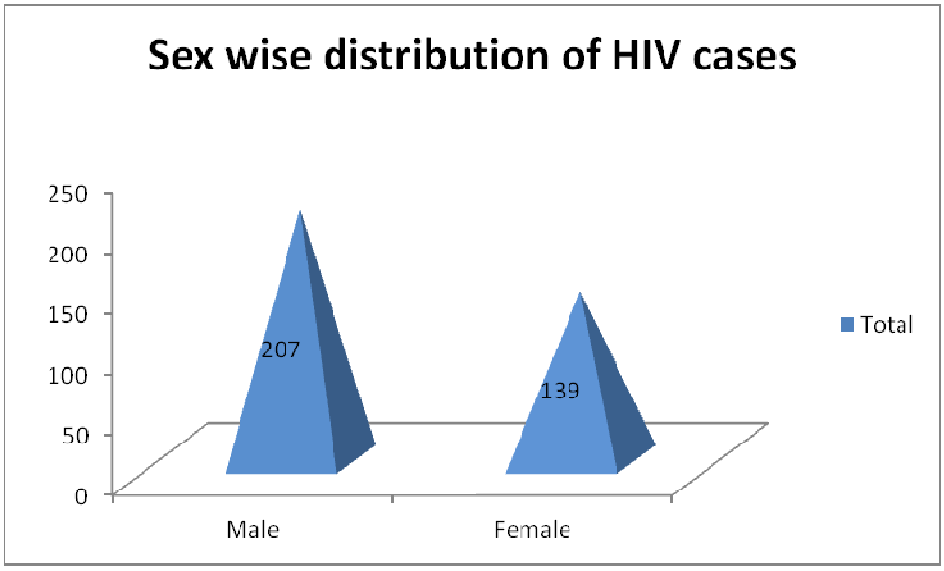


Chart - 3

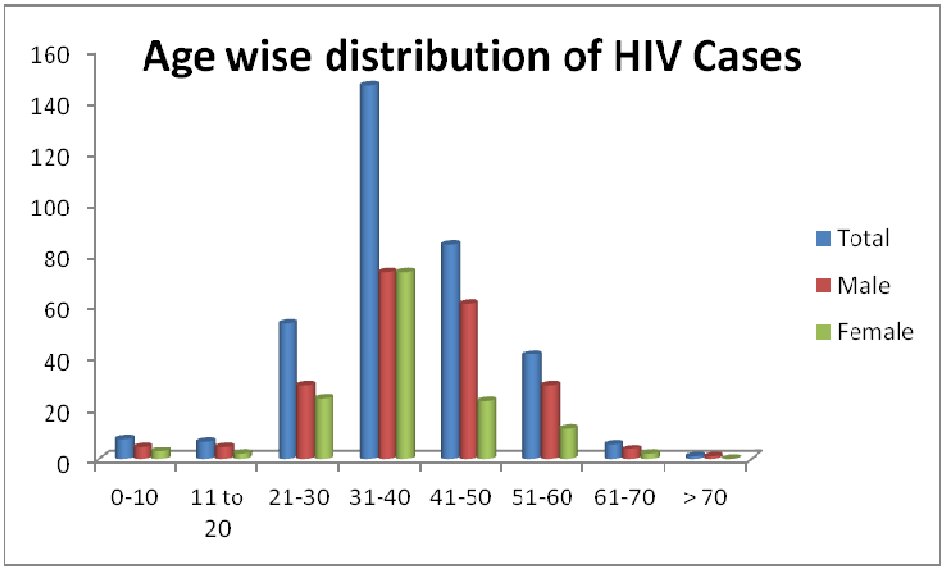


TABLE – 4

HABITAT WISE DISTRIBUTION OF HIV CASES

S.NO	RURAL/URBAN	TOTAL	MALE	FEMALE
1.	Rural	267	159	108
2.	Urban	79	48	31

Majority of the affected cases belong to rural areas.

TABLE – 5

EDUCATION WISE DISTRIBUTION OF HIV CASES

S.NO	EDUCATION	TOTAL	MALE	FEMALE
1.	Child	7	4	3
2.	Illiterate	113	60	53
3.	Primary	123	79	44
4.	Secondary	68	37	31
5.	Graduate	35	27	8

Majority of the affected cases are educated upto primary level i.e, low educational status.

Chart - 4

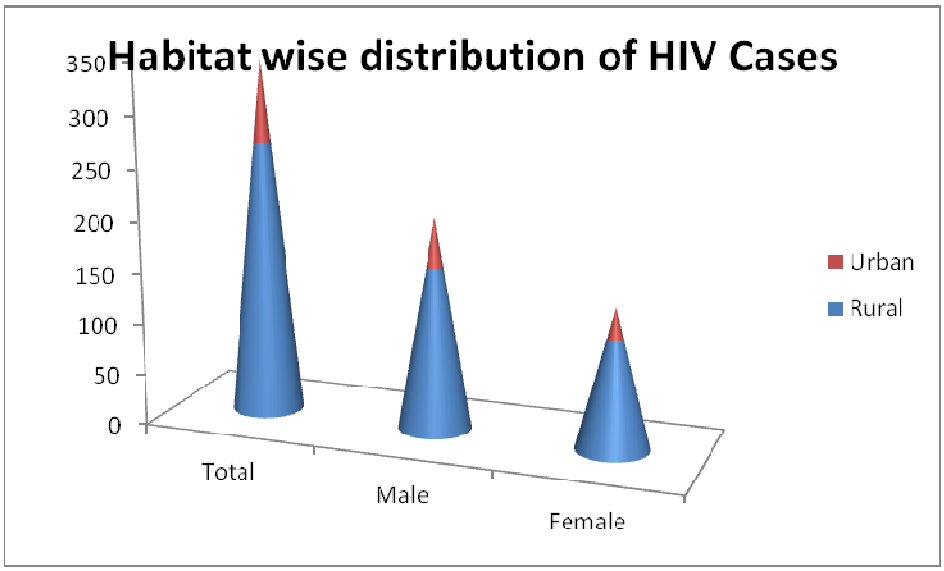


Chart - 5

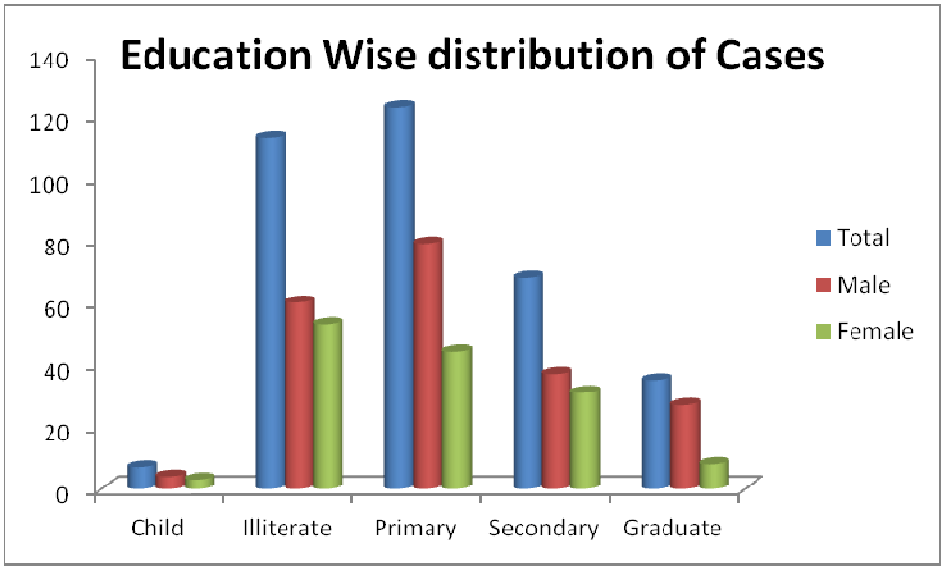


TABLE – 6

OCCUPATION WISE DISTRIBUTION OF CASES

S.NO	OCCUPATION	TOTAL	MALE	FEMALE
1.	Farmer	75	73	2
2.	Laborer	71	69	2
3.	Driver	36	36	0
4.	Service	21	15	6
5.	Business	8	6	2
6.	Ex military personnel	1	1	0
7.	House wife	123	0	123
8.	Child	7	4	3

Majority of the affected males are farmers and laborers and majority of the affected females are house wives.

TABLE -7

MARITAL STATUS WISE DISTRIBUTION OF CASES

S.NO.	MARITAL STATUS	TOTAL	MALE	FEMALE
1.	Married	312	182	130
2.	Unmarried	21	19	2
3.	Divorced	2	0	2
4.	Widow	2	0	2
5.	Widower	1	1	0
6.	Child	8	5	3

Married Cases fall under majority.

Chart - 6

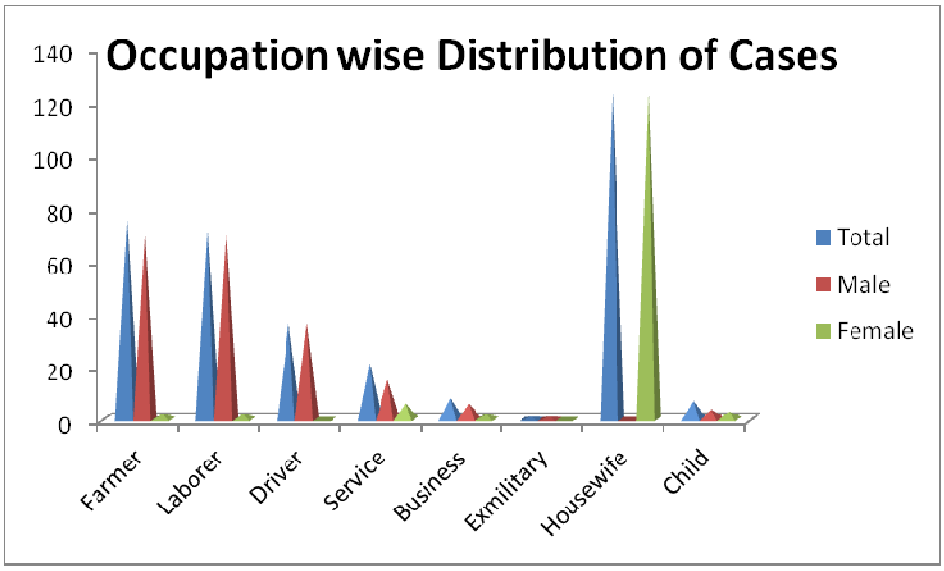


Chart - 7

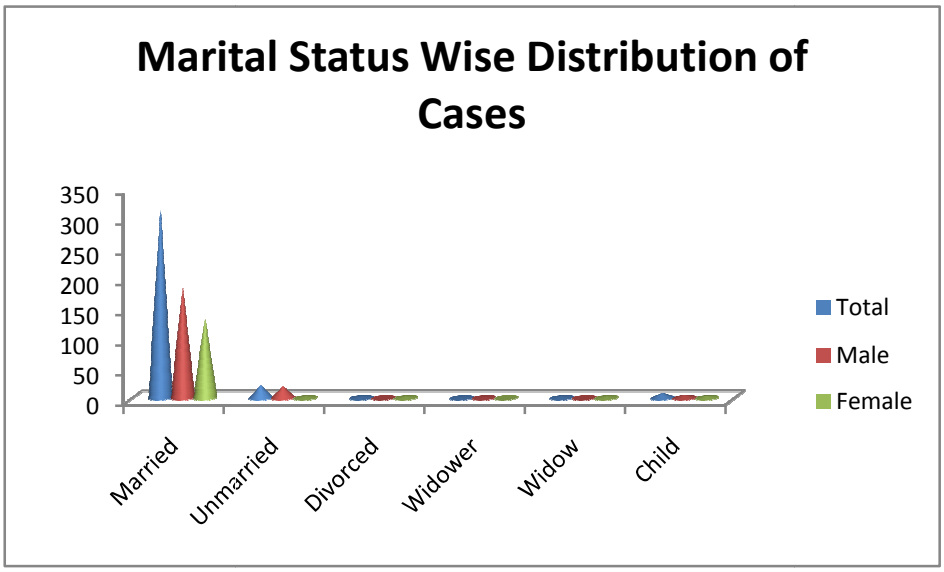


TABLE -8

ROUTE OF TRANSMISSION

ROUTE OF TRANSMISSION	TOTAL	MALE	FEMALE
Heterosexual	336	200	136
Perinatal	8	5	3
Blood transfusion	2	2	0

The major mode of transmission is heterosexual.

TABLE – 9

CD4 COUNT OF HIV REACTIVE CASES

S.NO.	CD4 COUNT CELLS/ML	TOTAL	MALE	FEMALE
1.	< 50	15	9	6
2.	51-100	23	16	7
3.	101-200	58	37	21
4.	201-350	80	52	28
5.	351-500	80	46	34
6.	> 500	90	47	43

Most of the affected males and females have CD4 count >500.

Chart - 8

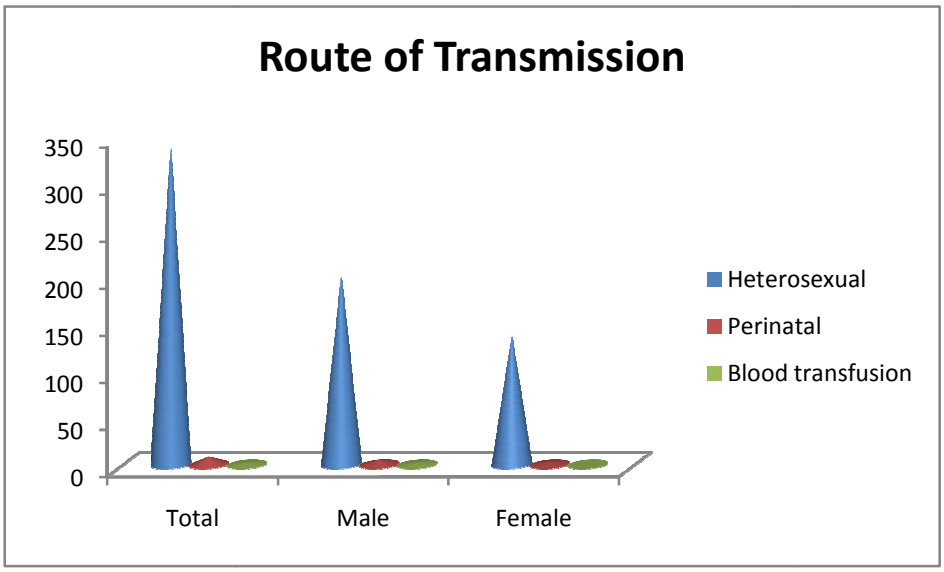


Chart - 9

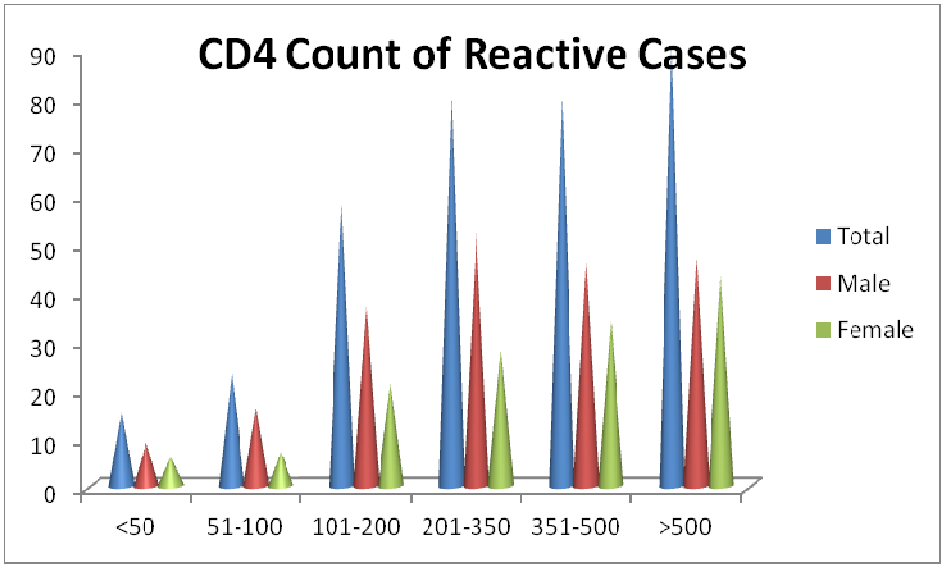


TABLE – 10

AGE WISE DISTRIBUTION OF ORAL CANDIDIASIS CASES IN HIV

S.NO.	AGE GROUP IN YEARS	TOTAL	MALE	FEMALE
1.	0-10	1	1	0
2.	11-20	1	0	1
3.	21-30	17	9	8
4.	31-40	55	26	29
5.	41-50	34	26	8
6.	51-60	21	15	6
7.	> 60	6	4	2

Majority of the affected males and females are in the age group of 31-40 years.

TABLE – 11

CD4 COUNT OF ORAL CANDIDIASIS CASES AMONG HIV CASES

S.NO.	CD4 COUNT CELLS/ML	TOTAL N=135	MALE	FEMALE
1.	< 50	14	10	4
2.	51-100	17	12	5
3.	101-200	52	33	19
4.	201-300	26	12	14
5.	301-500	24	13	11
6.	> 500	2	1	1

Majority of the cases with oral thrush have CD4 count between 101 -200.

Chart - 10

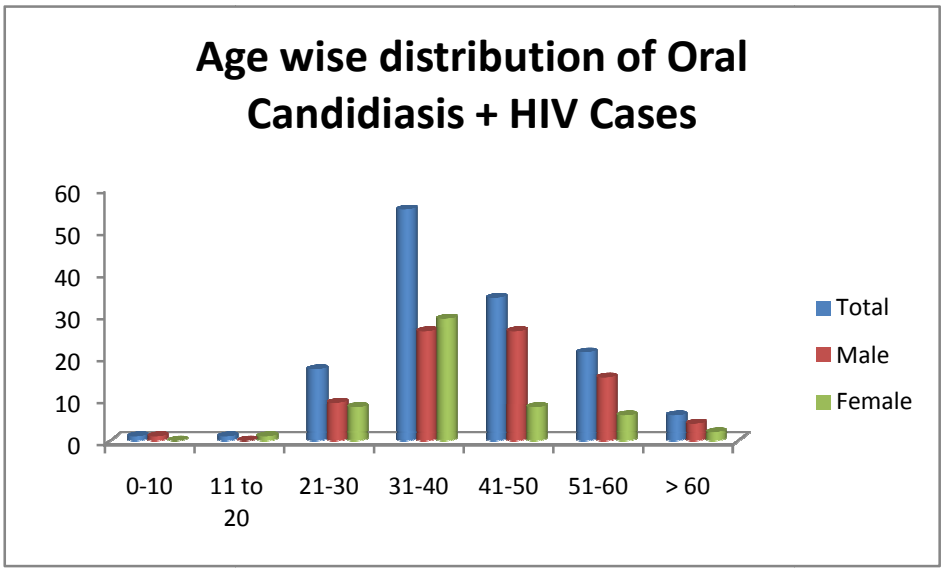


Chart - 11

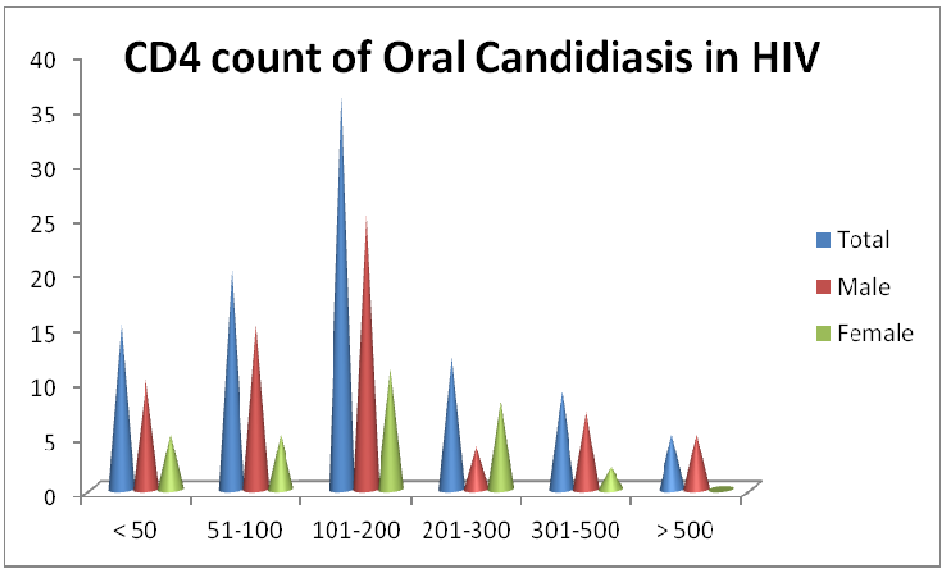


TABLE – 12

AGE WISE DISTRIBUTION OF PULMONARY TUBERCULOSIS CASES IN HIV.

S.NO	AGE GROUP IN YEARS	TOTAL N=97	MALE	FEMALE
1.	0-10	1	1	0
2.	11-20	2	1	1
3.	21-30	9	6	3
4.	31-40	47	29	18
5.	41-50	19	16	3
6.	51-60	18	13	5
7.	> 60	1	0	1

Majority of the affected males and females are in the age group of 31 to 40 years.

TABLE -13

CD4 COUNT OF PULMONARY TB CASES IN HIV.

S.NO.	CD4 COUNT CELLS/ML	TOTAL	MALE	FEMALE
1.	< 50	15	10	5
2.	51-100	20	15	5
3.	101-200	36	25	11
4.	201-300	12	4	8
5.	301-500	9	7	2
6.	> 500	5	5	2

Majority of the affected cases have CD4 count between 101-200.

Chart - 12

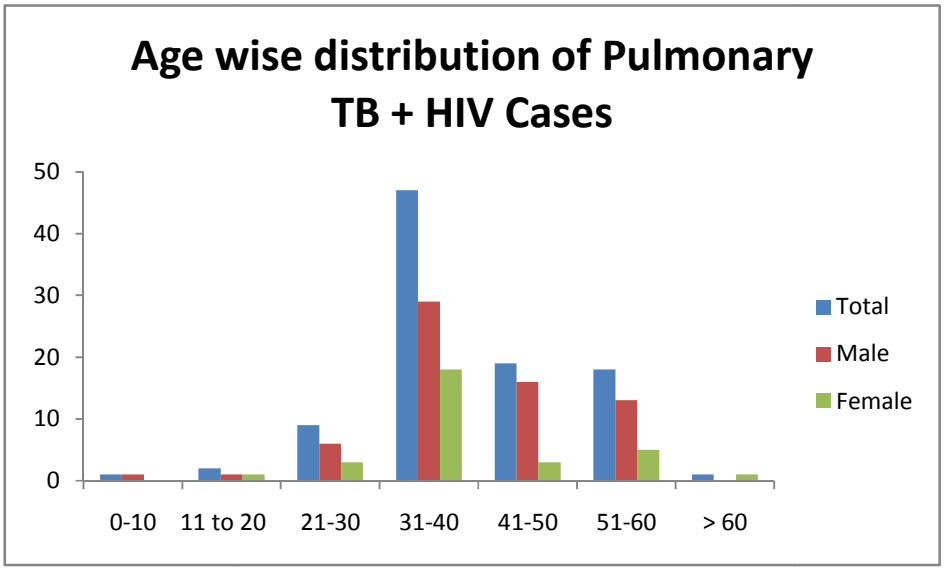


Chart - 13

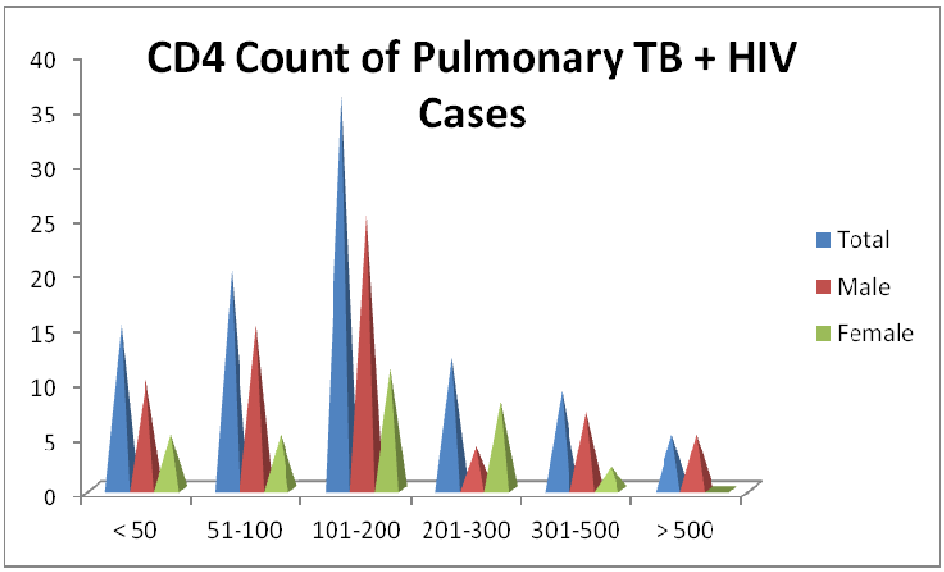


TABLE -14

AGE WISE DISTRIBUTION OF HSV-2 CASES IN HIV.

S.NO.	AGE GROUP IN YEARS	TOTAL	MALE	FEMALE
1.	0-10	-	-	-
2.	11-20	1	1	0
3.	21-30	1	1	0
4.	31-40	30	16	14
5.	41-50	10	9	1
6.	51-60	7	5	2
7.	> 60	1	1	2

The prevalence of HSV-2 in HIV increases with increasing age with majority falling in the age group 31 to 40 years.

TABLE – 15

CD4 COUNT OF HERPES SIMPLEX-2 CASES IN HIV.

S.NO.	CD4 COUNT CELLS/ML	TOTAL N=50	MALE	FEMALE
1.	< 50	2	2	0
2.	51-100	4	2	2
3.	101-200	24	15	9
4.	201-300	15	9	6
5.	>300	5	5	0

Majority of the HSV-2 + HIV infected have CD4 counts between 101 – 200.

Chart - 14

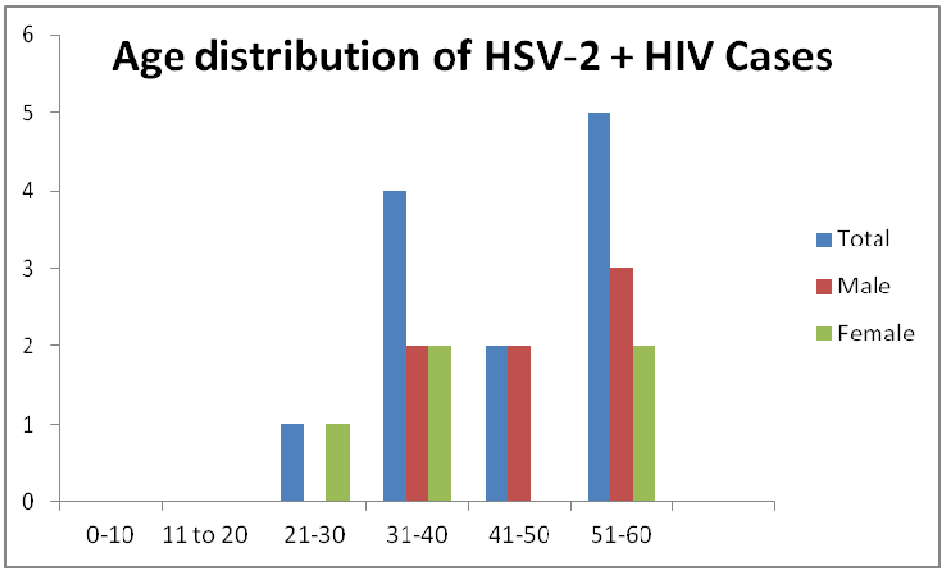


Chart - 15

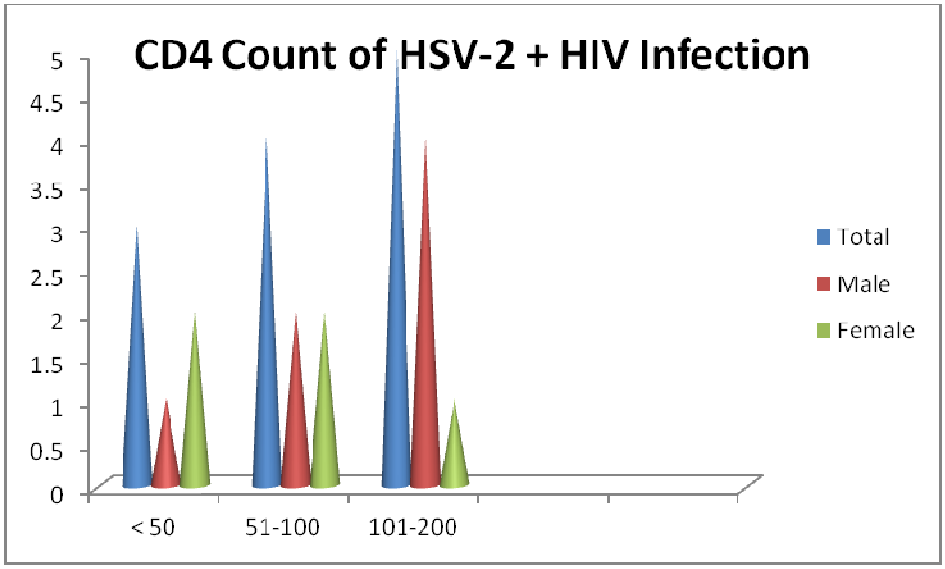


TABLE – 16

AGE WISE DISTRIBUTION OF TOXOPLASMOSIS CASES IN HIV.

S.NO.	AGE GROUP IN YEARS	TOTALN=20	MALE	FEMALE
1.	0-10	-	-	-
2.	11-20	-	-	-
3.	21-30	2	2	0
4.	31-40	8	4	4
5.	41-50	4	3	1
6.	51-60	5	5	0
7.	> 60	1	1	0

Majority of the cases are in the group of 31 to 40 years.

TABLE – 17

CD4 COUNT OF TOXOPLASMOSIS CASES IN HIV.

S.NO.	CD4 COUNT CELLS/ML	TOTAL N=20	MALE	FEMALE
1.	< 50	2	1	1
2.	51-100	3	3	0
3.	101-200	6	5	1
4.	201-300	9	6	3

Majority of the affected cases have CD4 count between 201-300.

Chart - 16

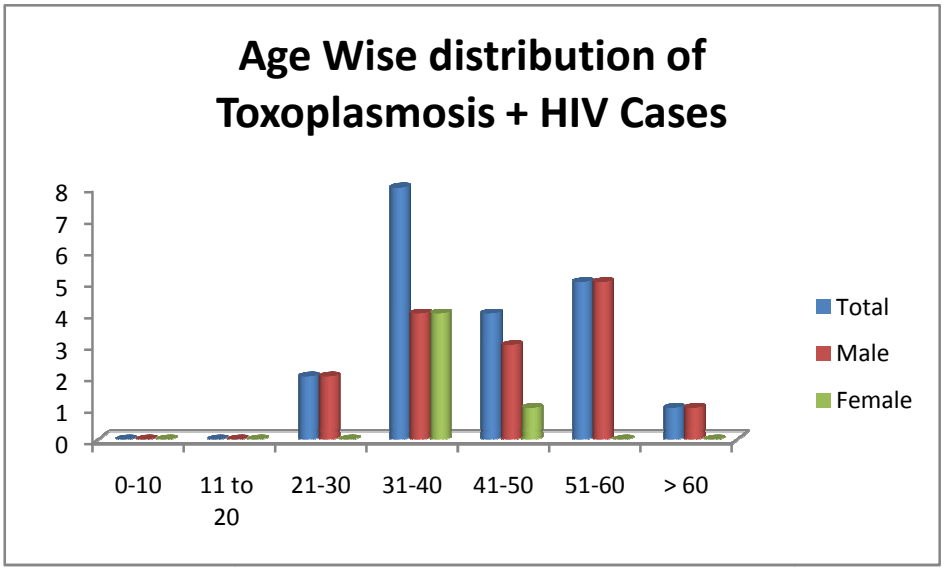


Chart - 17

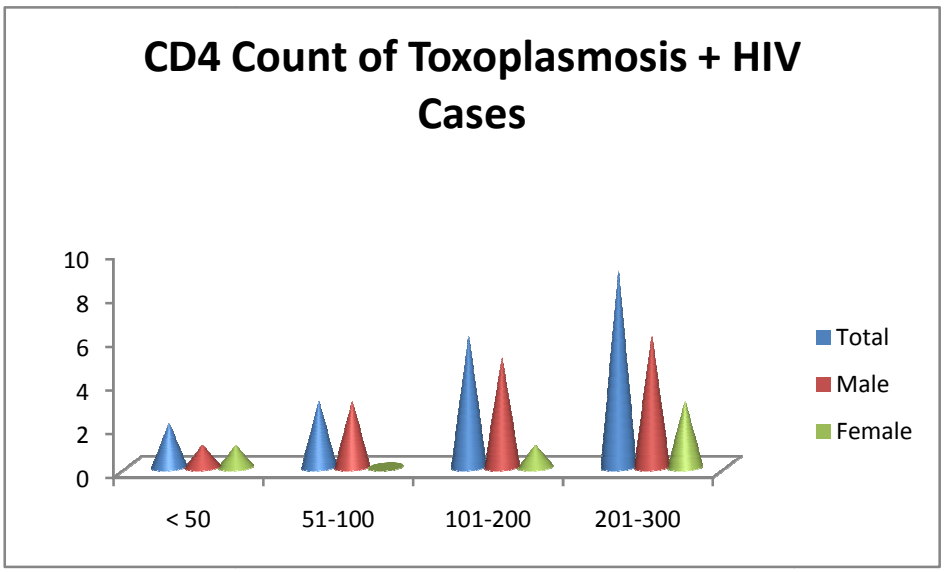


TABLE – 18

AGE WISE DISTRIBUTION OF CRYPTOCOCCOSIS CASES IN HIV.

S.NO	AGE GROUP IN YEARS	TOTAL	MALE	FEMALE
1.	0-10	-	-	-
2.	11-20	-	-	-
3.	21-30	1	0	1
4.	31-40	4	2	2
5.	41-50	2	2	0
6.	51-60	5	3	2

Majority of the affected cases are in the age group of 51-60 years.

TABLE – 19

CD4 COUNT OF CRYPTOCOCCOSIS CASES IN HIV.

S.NO.	CD4 COUNT CELLS/ML	TOTAL	MALE	FEMALE
1.	< 50	3	1	2
2.	51-100	4	2	2
3.	101-200	5	4	1

Majority of the affected cases have CD4 count between 101 to 200.

Chart - 18

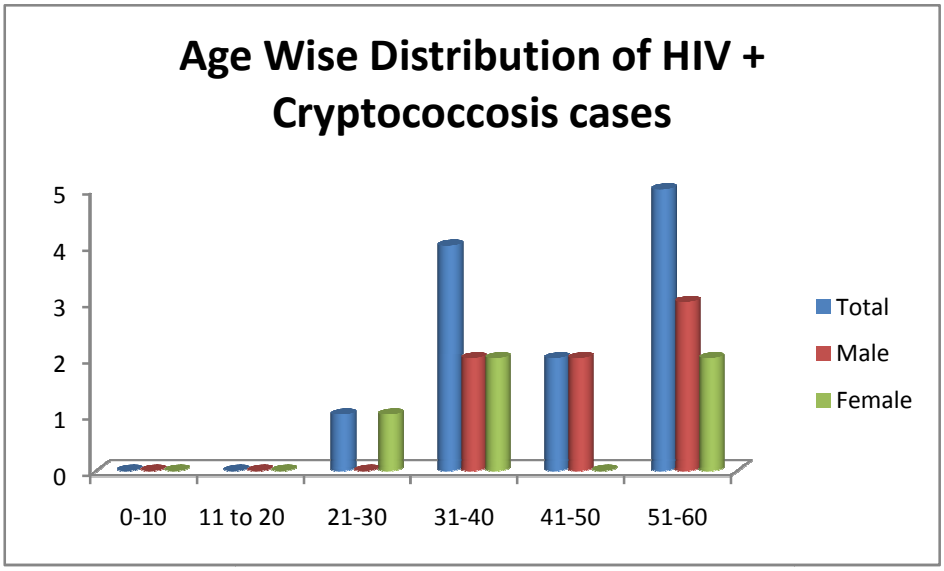
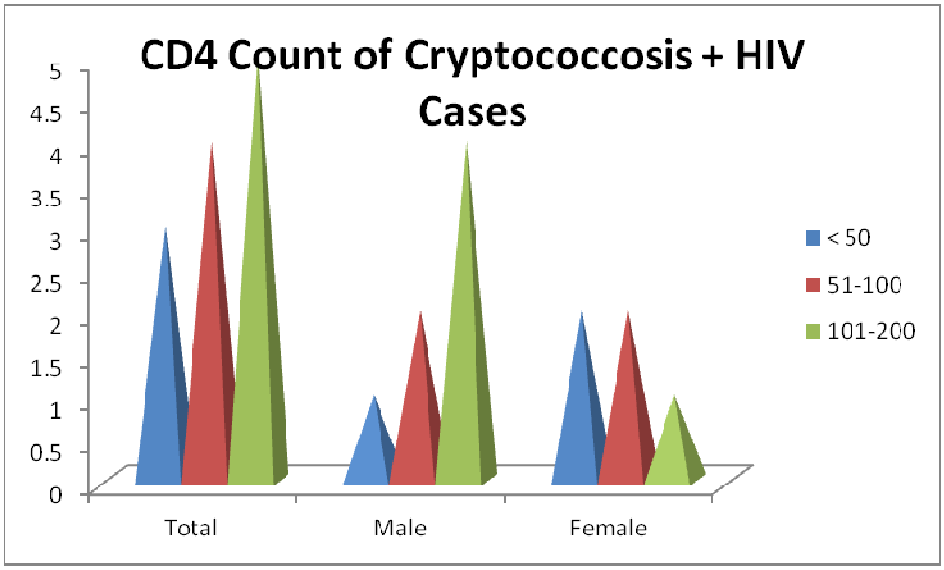


Chart - 19



DISCUSSION

Infection with HIV imposes heavy suffering on the affected individuals and in developing countries like India it imposes a great burden on the economy. All the health problems in HIV are due to waning immunity and the opportunistic infections in HIV are significant causes of mortality and morbidity and they pose a great challenge to the era of modern medicine having a great impact on the quality of life in HIV affected individuals.

Due to its non curable status, AIDS continues to be a major health problem. The primary medical care in India for HIV mainly consists of supportive treatment for symptoms, prophylaxis & treatment of Opportunistic infections. So early diagnosis of HIV and its opportunistic infections is vital. This study was done with 346 reactive cases in Thanjavur and the prevalence is about 2.8% which is high when compared to the overall prevalence of HIV in Tamil Nadu which is about 0.47%. The high prevalence of HIV in Thanjavur makes it necessary to evaluate the seropositivity, the immune status and the spectrum of Opportunistic Infections (56).

AGE WISE PREVALENCE :

In my study majority of the patients were in the age group of 31 to 40 years followed by 41 to 50 years. This observation matches with that of other workers and that of NACO. Nilanjan Chakrabothy et al, ICMR unit, Kolkata has shown that majority of them were in the age group of 31 to 40 years followed by 21-30 years. Aruna Agarwal et al has shown that most of the cases were in the reproductive age group. Similar observation is shown by Jagdish et al. Kesav Singh et al has shown that majority of them are in reproductive age group of 15 to 45 years (9, 35, 43, 62).

SEX WISE PREVALENCE :

In my study, majority(60%) are males. A study by NACO has shown males predominate with 61%. This observation matches with studies of Aruna Agarwal et al and Kesav Singh et al and Sarna A et al (9, 43). Nilanjan Chakrabothy et al has shown in his study that 84% were males and 16% were females. But in a study by Akinsegun Akinbomi et al(3) done in Nigeria has shown that 56% were females and 44% were females with a male female ratio 1: 1.8 with a female preponderance.

A majority of the subjects were from rural areas about 77.17% and 22.83% were from urban areas. Similar results are observed by NACO, Aruna Agarwal et al and Kesav Singh et al and Sarna A et al. Nilanjan Chakrabothy et al. This shows that awareness about AIDS is very much low in rural areas. Vyas N et al in his study done in North west India has also shown similar observations.

EDUCATION WISE PREVALENCE :

Most of the subjects were educated upto primary level (35.55%) followed by illiterates (32.66%). This indicates that most of the subjects have a low educational status. This is similar to the study conducted by Jayaram et al. But in a study by Kesav Singh et al done at Rewa district of Madhya Pradesh most of them were educated upto secondary level. In my study 56% of females were illiterate which is in contrast to a study by Gupta et al where 28% of the females were illiterate(29, 38, 43).

OCCUPATION WISE PREVALENCE :

Occupation wise majority were farmers(21.68%) and laborers (20.52%) who may indulged in risky activities because of illiteracy and lack of awareness. Similar observations are shown by most other workers. Among the males, 10.4% were drivers whereas in a study by Kesav Singh et al 21.68% were drivers and Vyas N et al has shown that 9.7% were drivers. Among the females, 88.49% were house wives. Kesav Singh et al has shown that 77.27% were house wives but a study done in Udippi has shown that only 44.5% were house wives (43,84).

MARITAL STATUS :

The distribution according to the marital status has shown that 90.17% were married and 6.07% were unmarried, 0.58% were widows and 0.29% were widowers. Among the males 87.92% were married and 9.18% were unmarried. Among the females 93.53% were married and 1.44% were unmarried. This is similar to the study done by Lal et al in North west India but Kesav Singh et al in his study has shown that 76.92% of males and 73.92% of females were married(43, 47).

ROUTE OF TRANSMISSION :

In my study the major route of transmission is heterosexual 97.11%, followed by perinatal transmission (2.31%) and blood transfusion (0.58%). Kesav Singh et al has shown heterosexual mode of transmission in 89.33%, perinatal transmission in 21%. Jagdish et al has shown heterosexual mode of transmission in 95.73%, perinatal transmission in3.4%. Nilanjan Chakrabothy et al has shown heterosexual mode of transmission in 80.33%, homosexual transmission in 6%, Injectable Drug Use in 16% and blood transfusion in 2.9% (35, 61).

CLINICAL PROFILE :

Fever(53%), weight loss(50%) followed by oral thrush (39%) and cough(30%) were the most common presenting features in my study which is supported by Aruna Agarwal et al who showed Fever(56%), weight loss(31%) followed by oral thrush (30%) and cough(50%) and Jagdish et al showed Fever(57%), weight loss(45%) followed by cough(18%). Anant A Takalkar et al showed Fever(85%), weight loss(65%) , cough(47%) and diarrhea in (41.77%) (4, 9,35).

CD4 STATUS :

Majority of the patients had CD4 counts > 500, followed by 200 to 500. Only about 30% of the patients had CD4 below 200. In my study CD4 status of the patients reveal that HIV is diagnosed at an earlier stage when the immune system is not much damaged. One more important finding is that the chances of getting opportunistic infection increases when CD4 falls below 200. In a study by Anant A Takalkar et al about 46.4% had CD4 < 200. Akinsen et al has shown that 42.4% had CD4 < 200. Whereas in a study conducted in New Delhi and in a study done by Gerardo et al most of the patients had CD4 < 200 reflecting late presentation of HIV (3, 4, 27).

OPPORTUNISTIC INFECTIONS – ORAL CANDIDIASIS :

In my study, oral candidiasis is the most common opportunistic infection 135 cases out of the 346 reactive cases (39.02%) had oral thrush. This finding correlates with the study of Shobana et al who has shown 36% of oral candidiasis and the study of Anant A Takalkar et al who has shown 39% of oral candidiasis and Aruna et al has shown a prevalence of 24.24% (9). In contrast, studies by Pande S et al, Nair S et al, Mulla S A et al has shown a low prevalence of

oral thrush of 22.7%, 16.5% and 19.4% respectively. Oral Candidiasis emerged as the most frequent infection associated with HIV (4, 54, 64, 72).

PULMONARY TUBERCULOSIS :

Pulmonary Tuberculosis emerged as the second common Opportunistic Infection following Oral thrush. 97 cases out of 346 cases (28.02%) showed Sputum smear positivity. But the most common presenting clinical feature in my study was fever (53%) and weight loss(50%) which can be attributed to Extrapulmonary Tuberculosis which is more common in HIV than Pulmonary TB and this entity was not studied. But in most studies which included EPTB, TB remained the first and foremost Opportunistic Infection. For example, studies by authors like Anant A Takalkar et al, Pande S et al, Zaheer M S et al, Singh A et al has shown a prevalence of 52.3%, 54.7%,57% , 56.0% respectively. But Jagdish et al has shown a prevalence of 25% which matches with my study. TB is highly endemic in India and multi drug resistance is common in HIV (4, 35, 50, 64, 73, 86).

HERPES SIMPLEX – 2:

The viral opportunistic infections in HIV/AIDS has become more common causing significant health problems in our community. Out of the 346 sera screened for HSV-2 IgG antibodies, 50 were seroreactive(14.45%). The low prevalence of HSV-2 in my study may be due to geographic and socio-economic factors. Nilanjan Chakrabothy et al(63) has shown a prevalence of 47% in his study.HIV infection is associated with increased risk of Human Herpes Viruses and their related disorders. Genital ulcers are the most common cause of disruption of epithelial barrier and infiltration of CD4 locally resulting easy and early acquisition of HIV. K Anuradha et al has shown a prevalence of 22%. Flemming et al and Varela et al has shown a

prevalence of 21% and 25% of HSV-2 in general population respectively(7). HSV-2 reactivation enhances the transmission of HIV. Immune deficiency in HIV decreases the inflammatory response of the host and therefore the lesions are not evident(28).

TOXOPLASMOSIS :

Out of the 346 sera screened for Toxoplasma IgG antibodies, 20 were reactive (5.78%). The prevalence of Toxoplasmosis in HIV varies from 3 to 97%. Studies have shown that the prevalence of Toxoplasma in USA is 10-40%, in Asia 10-50% and in Europe it varies from 4-90%. The increased prevalence of Toxoplasmosis may be due to behavioural changes induced by the parasite (25). In the pre-ART era, toxoplasmosis presented mostly as encephalitis but now disseminated forms involving lungs, eyes and spinal cord are common. This is the most important cause of Neurological Immune Reconstitution Inflammatory Syndrome(15, 25, 53, 76).

CRYPTOCOCCOSIS :

Out of the 346 sera screened for Cryptococcosis by Latex Agglutination Test, 12 were reactive (3.41%). It is caused by inhalation of spores of the fungus. The infection remains dormant. When immunity falls in case of HIV and CD4 count falls below 100, the infection is reactivated and spreads. Usually presents as meningitis and half of them die. The case load is high in Subsaharan Africa followed by South east Asia. The incidence of Cryptococcal meningitis varies. It is 3.6% in U.K., 4.5% in France, 6% in USA and 3% in India. It is a common Opportunistic infection in HIV but remains undiagnosed due to lack of awareness. Mostly it presents as chronic meningitis but can present as acute meningitis also. So Cryptococcal meningitis should be ruled out in any HIV patient presenting with nausea and

vomiting. WHO released “Rapid Advice” guidelines for diagnosis, prophylaxis and management of Cryptococcosis (30,77).

AMPLICOR HIV-1 DNA PCR :

10 samples were run and all the samples(100%) answered positive. Satarupa Sengupta et al has shown 100% positivity in her study done in Kolkata. JCM accepts study has shown a positivity of 93.3%. One sample answered negative due to low viral load. The quantification of HIV-1 DNA by nucleic acid based methods like PCR is necessary for evaluating the efficacy of ART therapy. It is also of great use in diagnosis of HIV in infants and adults in window period(23, 39, 69).

LIMITATIONS OF STUDY:

1. Opportunistic Infections other than Oral Candidiasis, Pulmonary Tuberculosis, Herpes Simplex-2, Toxoplasmosis and Cryptococcosis like Extrapulmonary Tuberculosis, Cryptosporidiasis, Pneumocystis jiroveci Pneumonia, CMV retinitis etc., were not studied due to lack of facility.
2. HIV-1 Subtyping was not done.
3. The study was limited to one centre.

SUMMARY

The present study was done at Thanjavur Medical College Hospital from September 2012 to September 2013 to find the seroprevalence of HIV and the spectrum of Opportunistic Infections associated with HIV.

- The sera of the patients were tested by HIV COMB-AIDS.
- The reactive sera were further tested by HIV Triline and HIV Trispot and the sera reactive by the three rapid tests were further confirmed by HIV Micro ELISA.
- Seroprevalence of HIV was 2.8%
- Preponderance of HIV was found more in males when compared to females and the common age group of the reactive cases was 31 to 40 years and majority were from rural areas and educated upto primary level.
- Majority of the males were farmers and most females were housewives.
- Majority of them were married and heterosexual route remained the predominant mode of transmission.
- One forth of the patients had CD4 count above 500 and one forth had CD4 counts < 200 cells. So most of them had good immune status at the time of diagnosis.
- Oral Candidiasis emerged as the most common Opportunistic Infection followed by Pulmonary Tuberculosis, Herpes Simplex Virus -2, Toxoplasmosis and Cryptococcosis.
- Molecular Characterisation was done for 10 samples by RT-PCR and all answered positive.

CONCLUSION

- This study highlights the epidemiological data and clinical presentation of HIV infection in and around Thanjavur.
- From this study, the role and the complex interrelationship between various social and demographic factors can be understood and thereby the transmission of HIV can be controlled and interrupted.
- The common age group of affected patients in this study is 31 to 40 years. This data highlights the need of intervention programmes like HIV awareness and safe sex education among the young adults.
- This study shows increasing trend of HIV infection spread among the house wives which has a direct impact on perinatal transmission and increased number of Paediatric AIDS. This has a powerful influence on the socio – economic and cultural development of a country.
- This study is aimed at providing base line data regarding the common Opportunistic Infections prevalent in our part thereby helps the physicians to take prompt therapeutic measures.
- Since most patients are diagnosed of HIV only when they present with Opportunistic Infections, a high level of alertness is needed both at the clinical and laboratory level and routine surveillance becomes mandatory.

Though curative treatment for HIV is not available at present, we can minimize the HIV infection by early screening and health education.

ANNEXURES

INFORMED CONSENT

I have been informed about the study on HIV infection. I am willing to give sample for the study, as I realize the importance of the study. I am also aware that I can withdraw from the study whenever I like.

Date : Signature of the patient

Department :

CLINICAL PROFORMA

Name of the patient: Serial No. :

Age : Lab No. :

Sex : OP/IP No. :

Address : Date Of Sample Collection :

Occupation : Income :

Chief Complaints :

1. Fever
2. Cough
3. White patches in oral mucosa
4. Loss of weight
5. Loss of appetite

Past History :

1. Tuberculosis 2. Bronchial asthma 3. Diabetes 4. Hypertension.

Family History :

Personal History :

1. Smoker 2. Alcoholic 3. Extra marital contact 4. Injectable Drug Use.

CLINICAL DIAGNOSIS :

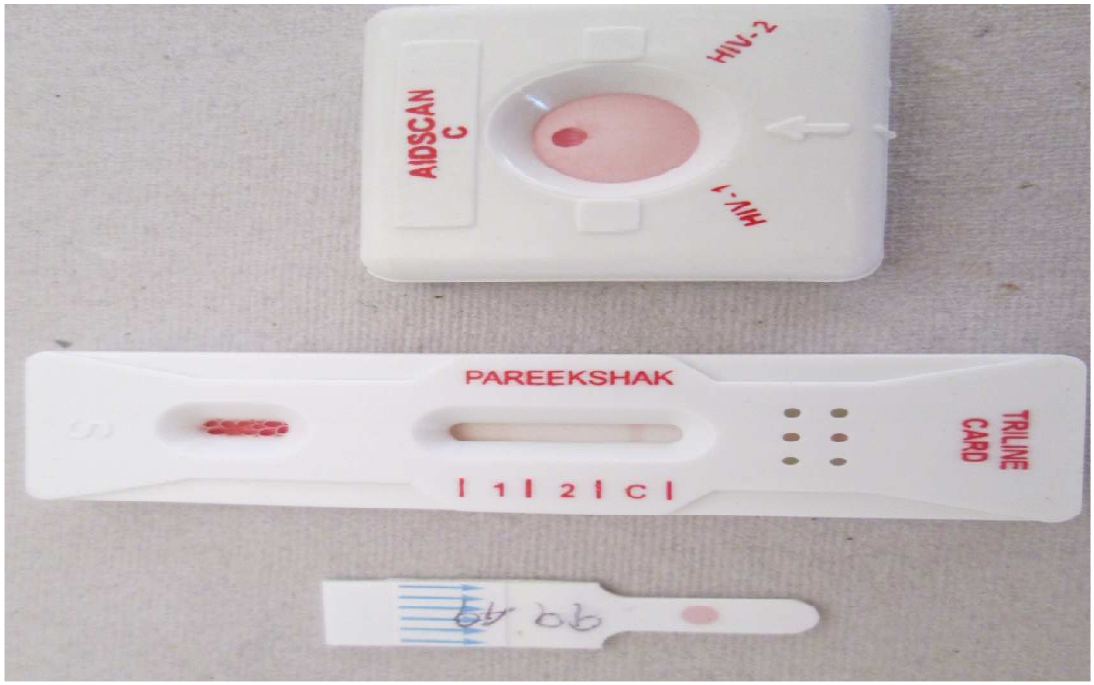
LABORATORY FINDINGS

1. HB%
2. WBC
3. PLATELET COUNT
4. HAEMATOCRIT %
5. LIVER ENZYMES
6. OTHER INVESTIGATIONS
7. HIV RAPID TEST :
8. HIV MICROLISA
9. MOLECULAR DIAGNOSIS
10. GRAM STAINING & CULTURE FROM ORAL THRUSH
11. SPUTUM AFB
12. HSV-2 IgG ELISA
13. TOXOPLASMOSIS IgG ELISA
14. CRYPTOCOCCAL LATEX AGGLUTINATION TEST :

Name	AGE/ Sex	HABITAT	EDUCATION	OCCUPATION	MARITAL STATUS	ROUTE	CD 4COUNT	TB, OC	HSV-2	CRY	TOXO
Aravindhan	18/M	rural	sec	student	nm	heterosexual	330	nil			
Madhumitha	7/F	rural				perinatal	432	nil			
Navab	43/M	rural	sec	labourer	m	heterosexual	87	TB, OC			
Vahidha	35/F	rural	Primary	HW	m	heterosexual	1032	nil			
Yelambal	55/F	rural	Illiterate	HW	divorced	heterosexual	40	TB, OC			
Ayyadurai	43/M	rural	Primary	labourer	m	heterosexual	370	OC			
Manimaran	24/M	urban	sec	driver	um	heterosexual	653	nil			
Uma	32/F	urban	sec	hw	m	heterosexual	471	nil			
Senthilkumar	34/M	rural	Primary	labourer	m	heterosexual	830	nil			
Rajamani	39/F	rural	Primary	farmer	divorced	heterosexual	95	TB, OC			
Indhira	40/F	rural	Illiterate	farmer	widow	heterosexual	175	TB, OC,	hsv		
Mahalakshmi	33/F	urban	graduate	hw	m	heterosexual	665	nil			
Saravanan	31/M	urban	sec	labourer	m	heterosexual	142	TB, OC	hsv		
Karthi	23/F	rural	sec	hw	m	heterosexual	389	OC			
P.Rani	45/F	rural	sec	hw	m	heterosexual	760	nil			
Rani	50/F	rural	sec	hw	m	heterosexual	131	OC	hsv		
Pitchaipillai	39/M	rural	Primary	farmer	m	heterosexual	100	Tb	hsv		toxo
Thangaraj	43/M	urban	graduate	service	m	heterosexual	108	oc, hsv	hsv		
Vimala	28/F	rural	sec	hw	m	heterosexual	720	nil			
Raman	28/M	rural	Primary	farmer	m	heterosexual	501	nil			
Ravi	28/M	rural	Illiterate	labourer	m	heterosexual	694	nil			
Indhirani	35/F	urban	Illiterate	hw	m	heterosexual	751	nil			
Mahalakshmi	35/F	rural	sec	hw	m	heterosexual	184	oc	hsv		
Rengan	30/M	rural	Illiterate	farmer	m	heterosexual	223	oc			toxo
Udayasankar	46/M	rural	sec	driver	m	heterosexual	313	nil			
Subbaiyan	57/M	rural	sec	farmer	m	heterosexual	564	nil			
Shyamala	24/F	urban	Primary	hw	widow	heterosexual	639	nil			
Sureshbabu	30/M	rural	sec	labourer	m	heterosexual	854	nil			
Mari	47/m	rural	Primary	farmer	widower	heterosexual	813	nil			
Sujatha	37/F	rural	sec	hw	m	heterosexual	231	oc	hsv		
Selvaraj	44/M	rural	Primary	driver	m	heterosexual	453	nil			

1. COMB AIDS, Triline, Trispot kits

2. Immuno chromatography – Non reactive for HIV



3. Immuno chromatography – Reactive for HIV-1

4. Immuno chromatography – Reactive for HIV-1 & 2

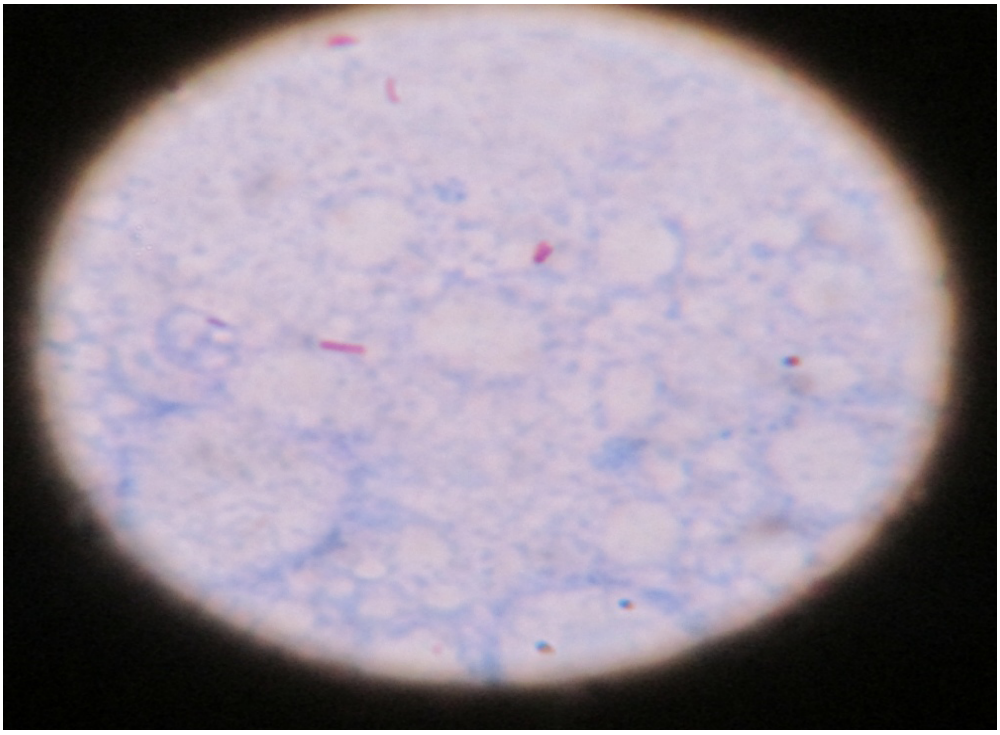
5. ELISA Reader

6. HIV Microlisa kit

7. HIV Microlisa – Test Results

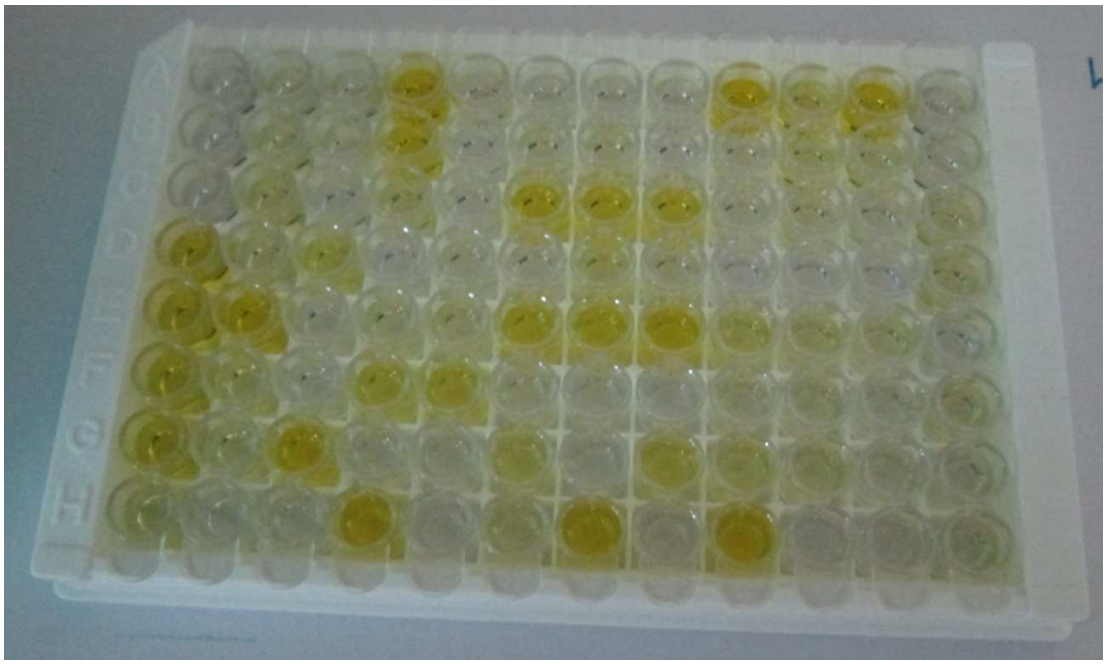
8. BD FACS Count System

9. Sputum AFB Positive



10. Candida growth in SDA.

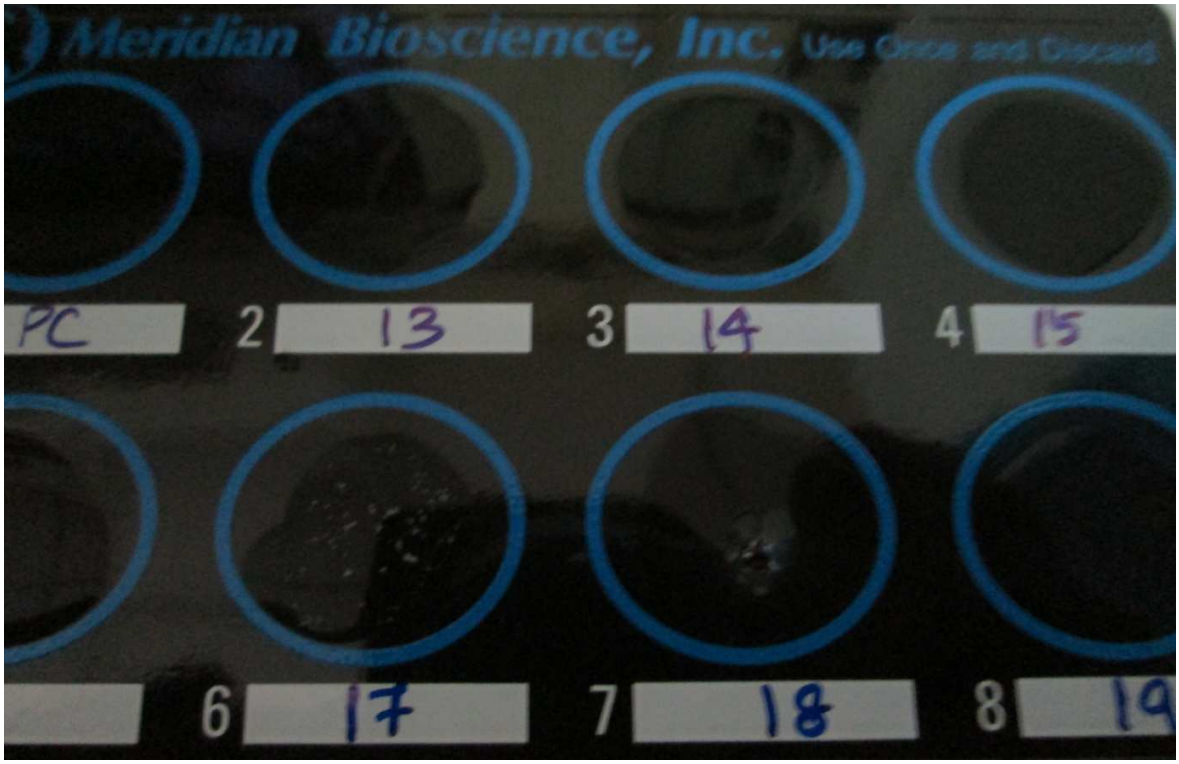
11. HSV-2 IgG ELISA – Test Results



12. Toxoplasmosis IgG ELISA kit

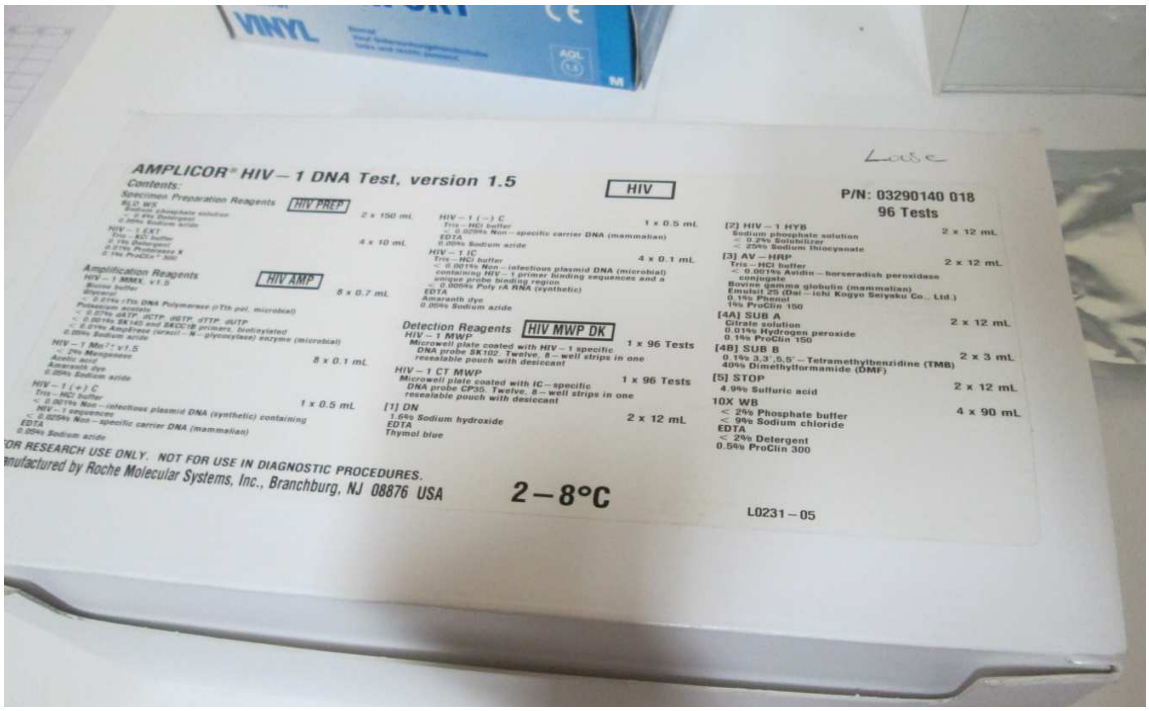
13. Cryptococcosis Latex Agglutination Kit

14. Cryptococcosis Latex Agglutination – Test Results



15. Thermal Cycler

16. AMPLICOR HIV-1 DNA PCR kit



BIBILIOGRAPHY

1. Afsar I, Gungor S, Sener AG, The prevalence of HBV, HCV and HIV Infections among blood donors in Izmir, Turkey. Indian Journal of Medical Microbiology. Vol.26, No. 3

2. Ajay M, Kumar V, Devesh Gupta, Will Adoption of 2010 WHO ART Guidelines for HIV-Infected TB Patients Increase the demand for ART Services in India? PLOS ONE September 2011, Volume 6, Issue 9.

3. Akinsegun Akinbami, Adedoyin Dosunmu, CD4 Count pattern and demographic distribution of Treatment- Naïve HIV patients in Lagos, Nigeria. AIDS Research and Treatment Vol 2012.

4. Anant A. Takalkar, Saiprasad, Study Of Opportunistic Infections in HIV seropositive patients admitted to Community Care Centre, KIMS, Narketpally. Biomedical research 2012;23(1): 139-142.

5. Ananthanarayanan and Paniker's Textbook of Microbiology, 8th edition, pp 569-573, University press publications.

6. Ananya Mandal, History of AIDS, News Medical, Maoyun Biochemical Technology, September 2013.

7. Anuradha K, Maan Singh H, Gopal KVT, HSV-2 Infection, a risk factor for HIV in heterosexuals.

8. Archana Sharma, Marfatia YS, Lab Diagnosis of HIV. Indian J Sex Transm Dis 2008, Vol.29, No.1

9. Aruna Agarwal, Usha Arora, Clinico-microbiological Study in HIV Seropositive Patients. JIACM 2005;6(2) 142-5.

10. Arun Kumar Jha, Beena Uppal, Clinical & Microbiological profile of HIV Cases with Diarrhoea in North India. J of Pathogens Vol.2012.

11. Ashish S. Bodhade, Sindhu M. Oral manifestations of HIV & their correlation with CD4 Count. J of Oral Science vol.53 No.2 203-211,2011.

12. Ayyagiri A, Sharma AK, Prasad KN et al. Spectrum of Opportunistic Infection in HIV infected cases in a tertiary care hospital. Indian J Med Microbiology 1999;17: 78-80.

13. Bailey and Scott Textbook of Diagnostic Microbiology 12th edition, pp 478-508, Elsevier publications.

14. Barrow G, Clarke TR, An analysis of three Opportunistic Infections in an Outpatient HIV Clinic in Jamaica. West Indian Med J 2010: 59(4): 393.

15. Basavaprabhu Achappa, Soundarya Mahalingam. Clinical spectrum & outcomes for Toxoplasma Encephalitis among AIDS patients before and during the era of ART in Mangalore, India.

16. Baveja CP, Textbook of Microbiology 3rd edition, pp 500-511, Arya publications.

17. Benefits of early ART therapy in patients with HIV-TB Coinfection. Indian J of Tuberculosis. Vol.56 No.3, July 2009.

18. Brian C Zanoni, Thuli Phungula, Predictors of poor CD4 & weight recovery in HIV-Infected children initiated on ART in South Africa. PLoS One March 2012, Vol.7, Issue 3.

19. Cauldbeck MB, O'Connor, Adherence to ART among AIDS patients in Bangalore. India AIDS Research & Therapy .2009;6:7

20. Chatterjee's Textbook of Parasitology 13th edition, pp 129-133, CBS publications.

21. Conie R. Mahonn's Textbook of Diagnostic Microbiology, 4th edition pp 573-603, Elsevier publications.

22. Darrel Hoi-San Tan, Kellie Murphy HSV-2 & HIV progression BMC Infectious Diseases 2013:502.

23. David S, Sachithanandham J, Comparison of HIV-1 RNA level estimated with plasma & DBS samples: A pilot study from South. IJMM 2012, 30(4): 403-6.

24. Field's Textbook of Virology, Vol.2, 5th edition, pp 2107-2214, Lippincott William Wilkins publications.

25. Fiseeha Walle, Nigahu Kebede, Seroprevalence & Risk factors for Toxoplasma in HIV infected and non-infected individuals in Bahir Dar North West Ethiopia. Parasites and Vectors 2013, 6:15.

26. Garcia Diaz A Clewley GS,Comparative Evaluation of the performance of the Abbott Real-Time HIV-1 Assay for measurement of HIV-1 Plasma Viral load following automated specimen preparation. JCM, May 2006 p1788-91

27. Gerardo Alvarez-Urin, Manoranjan Midde, Factors associated with late presentation of HIV & Estimation of ART need according to CD4 Count. Interdisciplinary Perspectives on Inf Diseases. Vol2012.

28. G Paz- Bailey, M Ramasamy, HSV-2 Epidemiology and management option in developing countries. STI 2007 Feb; 83(1): 16-22.

29. Gupta M, A profile of clients tested HIV positive in VCTC of district hospital in Udippi. Ind J Com Medicine 2009: 34(3) : 223-26.

30. Harapriya Kar, Gurjeet Singh, Cryptococcal Meningitis & HIV. World J of Medicine & Medical Science. Vol.1, No.2 June 2013 pp 37-39

31. Harrison's Textbook on Infectious Diseases, 17th edition, pp 792-895, McGrow Hill publications.

32. HIV/AIDS Scenario in India. www.delhi.gov.in.

33. Holmes HC, Davis CL, Report of an International Collaborative Study to evaluate a WHO HIV-1 RNA genotype Reference Panel. WHO 2003.

34. Imade PE, Eghafona NO, Prevalent Opportunistic infections associated with HIV positive children 0-5 years in Benin city, Nigeria. Malaysian J of Microbiology, Vol.4(2) 2008, pp11-14.

35. Jagdish C, Varsha G, Clinico-epidemiological profile of HIV infection over period of 3 years in a north Indian tertiary care hospital. IJMM Vol.31, No.3

36. Jagdish Chander's Textbook of Medical Mycology- 3rd edition, pp 266-314, Mehtha publications.

37. Jawetz Textbook of Medical Microbiology, 22nd edition, 516-521, Mcgraw Hill publications.

38. Jayaram S, Shenoy S, Profile of attendees at a VCTC of a hospital in Costal Karnataka. Ind J of Com Medicine 2008; 33: 43-46.

39. JCM Accepts, J. Clin. Microbiology JCM 01516-12.

40. John T Brooks, Jonathan E. Kaplan, HIV-associated Opportunistic Infections- Going, Going, But not gone. HIV-associated Opportunistic Infections. CID 2009:48 March 1.

41. Jonathan Cohen's textbook on Infectious Diseases, Vol.2, 3rd edition, pp 944-88, Mosby publications.

42. Kannangai R, David S, HIV-2 – A milder, kinder virus: An update.

43. Kesav Singh, Karuna Jindwani Clinical & demographic profile of newly detected HIV positive patients registered at ART Centre of a Medical College. J of Clinical & Diagnostic Research, 2012, Sep. Vol6(7)1167-70.

44. Koneman's Color Atlas & Textbook on Diagnostic Microbiology, 6th edition, pp 1065-1117, Lippincot William Wilkins publications.

45. Kuby's Textbook on Immunology, 6th edition, pp 161-163, Freeman publications.

46. Kulkarni SK, Doibale MK, Trend of seroprevalence of HIV among Antenatal clinic attendees at a tertiary care hospital. International J of Basic & Applied Med Sciences vol.2013(1) pp 257-262.

47. Lal S The surveillance of HIV/AIDS in India. Indian J of Community Medicine.

48. Mackie & McCartney's Textbook on Practical Medical Microbiology, 14th edition, pp 632-654, Elsevier publications.

49. Mandell's Textbook on Tropical Medicine, Vol.2, 7th edition, pp 2323-2333, Elsevier publications.

50. Manisha Ghate, Swapna Deshpande, Incidence of common Opportunistic Infections in HIV-infected individuals in Pune, India. International J of Infectious Diseases 2009, 13 e1-e8.

51. Margaret Fearon, The lab diagnosis of HIV Infections. Can J inf Med Microbiology Vol.16 No.1 Jan/Feb 2005.

52. McCullough MJ, Firth NA, HIV infection: A review of mode of infection, pathogenesis, general & clinical manifestations. Australian Dental Journal 1997; 42(1) 30-7.

53. Minco Mehraz, Farhad Mehrkani. Seroprevalence of Toxoplasmosis in HIV/AIDS patients in Iran.

54. Mulla SA, Patel MG, Study on Opportunistic Infections in HIV seropositive patients. Ind J of Com Medicine 2007: 32(3); 208-209.

55. Murugan S, Anburajan R, prevalence of HIV-2 infection in South Tamil Nadu, Ind J Sex Transm Dis 2007; Vol 28, No.2

56. NACO The NACP Phase III (2007-2012): Strategy & Implementation plan; Ministry of Health & Family Welfare; Govt. of India;2006.

57. Nair SP, Moorthy KP, Clinico-epidemiological study of HIV patients in Trivendrum. Ind J of Derm Ven Lep 2003; 69 ; 100-103.

58. Namita Jaggi's Textbook on Micrbiology for MLT, 1st edition, pp 260-278, Jaypee publications.

59. Narayan Srihari, Kumudhini, The CD4 count recovery in HIV and TB coinfected patients versus TB uninfected HIV patients in a tertiary care hospital. JPBMS vol.12, issue 13.

60. Nayana A. Ingole, Seema M. Kukreja, Role of HIV-1 Viral load in initiating ART. World J of AIDS, 2011, 1, 149-154.

61. Nilanjan Chakrabothy, Anirban Mukherjee, Current trends of Opportunistic Infections among HIV seropositive patients from Eastern India. Jpn. J. Inf. Dis., 61, 49-53, 2008.

62. Nilanjan Chakrabothy, ICMR Virus Unit, Kolkata, HIV- Co opportunistic Infections – A current picture in Tropical Climatic Eastern Indian seropositive population.

63. Nilanjan Chakrabothy, Sohinee Bhattacharyya, Incidence of multiple Herpes infection in HIV seropositive patients, a big concern for Eastern Indian Scenario. Virology J 2010,7:147

64. Pande S, Sundar S, Clinical profile & Opportunistic Infections in HIV/AIDS patient attending S.S Hospital Varanasi. Ind J Prev. Soc. Medicine 2008;39; 1-2.

65. Parija SC Textbook on Microbiology & Immunology, pp 589-601, Elsevier publications.

66. Raja K, Chandrasekar C, Zero CD4 count : A case of discordant CD4 response in a patient with well suppressed viral load. IJMM vol.31 No.3.

67. Richard Guerrant, David Walker's textbook on Tropical Infectious Diseases, Vol.2, 3rd edition pp 541-558, Elsevier publications.

68. Samir Lakshmi, Madhuri Thakkar, HIV Infection in India : Epidemiology, Molecular epidemiology & pathogenesis. J. Biosci. 33(4), Nov 2008 515-25

69. Satarupa Sengupta, Smarajit Jana, Phylogenetic analysis of p24-p7 region of the HIV-1 gag gene to determine subtype distribution among Female sex workers in Calcutta, India, JCM Nov.2005, p5787-5791.

70. Shallu Rozra, Debabrata Kundu, Periodontal status of HIV infected patients with special reference to CD4 count in West Bengal India, Asian Pacific J. of Tropical Disease 2012;2(6): 470-474.

71. Shashi Kumar BM, Harish MR, CD4 count a monitoring tool in HIV webmedcentral.com

72. Shobana A, Guna SK, Mucocutaneous manifestations of HIV infections Ind J of Derm Ven Leprology 2004; 70(2): 82-86.

73. Singh A, Bairy I, Spectrum of Opportunistic Infections in AIDS cases Ind J Med. Sci 2003; 57; 16-21

74. Smirti Naswa, Marfatia YS, Microbicides & HIV ; A review and update. Ind J of Sexually Transm Dis & AIDS 2012 vol.33 No.2

75. Sravya Kurapathi, Madhu Vajpayee Adolescents living with HIV: an Indian profile, AIDS Research & Treatment vol.2012

76. Sucilathangam G, Palaniapan N, Serological surveillance of Toxoplasmosis in a district in Tamil Nadu, IJMR 137, March 2013, pp 560-563.

77. Sushell Kumar, Ajay Wanchu, Cryptococcal meningitis in HIV. Neurology India Oct-Dec 2008 vol.56 issue 4.

78. Swati gupta, Richa Gupta, Seroprevalence of HIV in pregnant women in north India; a tertiary care hospitsl based study. BMC Inf Dis 2007 7:133

79. Teja VP, Sudha T, Cases and pattern of mortality in HIV infection of hospitalized patients in a tertiary care hospital, I J of Med Sci 2007: 61(10) ; 555-561.

80. Topley & Wilson's Textbook of Virology, Vol.2, 10th edition, pp1322-1344, Hodder Arnold publications.

81. Tripathi KD textbook on Essentials of Medical Pharmacology, 6th edition, pp 770-776, Jaypee publications.

82. Tripathi P, Agarwal S Immunobiology of HIV Infection www.ijmm.org.

83. Ujjwal Neogi, Irene Bontell, Molecular Epidemiology of HIV-1 subtypes in India ; Origin & Evolutionary history of predominant subtype C. PLoS ONE Jun 2012 Vol.7 issue 6

84. Vyas N, Haja S, The prevalence of HIV/AIDS and prediction of future trends in North west region of India. IJ of Com Medicine 2009: 34(3) 212-17

85. Wanchu A, Kuttiatt VS, CD4 count recovery in HIV/TB coinfected patients versus TB uninfected HIV patients. I J of Pathology & Microbiology 53(4) Oct-Dec 2010.

86. Zaheer MS, Rabbani MU, Clinical & Demographic profile of HIV patients in & around Aligarh J of Ind Acad Clin Medicine 2003; 4(2); 121-126.